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SENSITIZATION OF THE SUBMAXILLARY GLAND TO ACETYLCHOLINE BY SECTION OF THE CHORDA TYMPANI

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Pierce and Gregersen (1937) sectioned the chorda tympani on one side in dogs, and two months later found that the submaxillary gland with cut nerve responded to intravenous administration of acetylcholine with less secretion than the opposite, normally innervated gland. They suggested that there may have been sensitization to acetylcholine at a shorter time after the operation. Recent work on the iris sphincter (Keil and Root, 1941) makes this possibility appear likely. The experiments presented here were undertaken to learn whether or not there is sensitization of the parasympathetically decentralized submaxillary gland to acetylcholine.

Cats were operated upon aseptically under ether anesthesia. The chorda tympani on one side was dissected out of the chorda-lingual trunk and sectioned as far centrally as possible. The nerve was then freed from the submaxillary duct as far toward the hilus of the gland as practicable, always to well beyond the mental limit of the sublingual gland. The lingual trunk was carefully freed of all connection with the submaxillary duct, to obviate the possibility of innervation of the gland by recurrent chorda fibers (cf. Langley and Anderson, 1894).

At different periods after this operation the animals were anesthetized by intraperitoneal injection of dial (Ciba), and the chorda-lingual trunk on the control side was sectioned. The submaxillary ducts of both sides were then cannulated. All branches of both external carotid arteries, except the anterior lingual arteries and the submaxillary branches of the external maxillary arteries, were tied off. A cannula was inserted into each anterior lingual artery, directed centrally. Acetylcholine in small volume (0.05 cc.) was injected at a uniform rate directly into the arterial supply of each submaxillary gland through these cannulae. The flow of saliva was registered by a drop counter similar to that of Gibbs (1927), each drop corresponding to 0.024 cc.

Table 1 shows the results of six experiments at various times after removal

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of the chorda supply of one submaxillary gland. The smallest amount of acetylcholine which would cause secretion of one drop of saliva was determined for both glands, and was denominated the minimal effective dose. The ratio of this value for the normal gland to that for the denervated gland yields the figures in the last column of the table. It will be seen that 10 days after severance of the chorda tympani there was slight sensitization of the submaxillary to acetylcholine, and that such sensitization increased somewhat with time. The effect persisted through the 27th day after operation.

TABLE 1

Minimal effective doses of acetylcholine for normal and parasympathetically decentralized submaxillary glands

DAYS SINCE PARASYMPATHETIC DECENTRALIZATION	MINIMAL EFFECTIVE DOSE OF ACETYLCHOLINE (MICROGRAMS)		$\frac{N}{D}$
	Normal (N)	Denervated (D)	
10	0.2	0.15	1.3
13	0.1	0.03	3.3
16	2.0	0.5	4.0
17	2.0	0.1	20.0
18	0.2	0.08	2.5
27	0.3	0.03	10.0

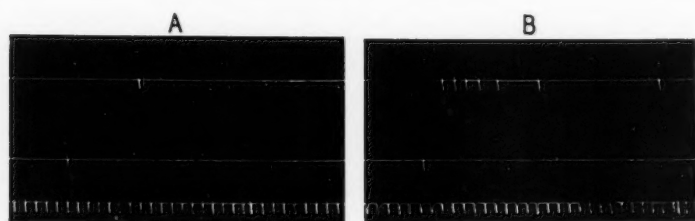


Fig. 1. Responses to 0.2γ of acetylcholine of normal (A) and parasympathetically decentralized (B) submaxillary glands of a cat operated upon 18 days previously (see table 1). Bottom line, time in 5 sec.; middle line, signal of injection; top line, drops of saliva (1 drop = 0.024 cc.).

It was found that the response of the submaxillary gland to acetylcholine was not directly quantal, and therefore table 1 does not demonstrate sensitization by parasympathetic decentralization so clearly as does figure 1. This figure records responses to the same dose of acetylcholine of the normal and denervated glands of the cat operated upon 18 days previously. The latter gland produced many times as much saliva as the former, although according to the figures for minimal effective doses of acetylcholine in table 1 sensitization was not marked.

There is evidence that in the cat outlying ganglion cells are scattered along the chorda tympani nerve as it nears the submaxillary hilus (Langley, 1890). Bradford (1888) had noted that in two of three cats excision of the chorda as

near as possible to the hilus resulted, after 3 to 6 days, in a loss of response of the gland to electrical stimulation. It is possible, therefore, that in some of the cats reported in table 1 the ganglion cells had been removed, i.e., that the submaxillary was denervated rather than being decentralized. It is known that denervation sensitizes to adrenaline to much greater degree than does decentralization. Possibly in the two animals in which the response was extreme (i.e., the two tested 17 and 27 days after operation) the gland was quite deprived of parasympathetic neurons. Whether the submaxillary was denervated or decentralized, however, the results clearly prove that it was sensitized to the action of acetylcholine.

SUMMARY

By severance of the parasympathetic nerve supply (section of the chorda tympani), the submaxillary gland of the cat was shown to be sensitized to acetylcholine, the effect being apparent 10 days after the operation and lasting at least through the 27th day.

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THE RATE OF EMPTYING OF THE RAT'S STOMACH FOLLOWING THE INTRAGASTRIC ADMINISTRATION OF GLUCOSE SOLUTIONS

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It has been suggested (1) that contractions of the stomach and its secretions influence the rate of glucose absorption from the intestine. This may be accomplished by controlling the rate of supply to the gut and by altering the pH, electrolyte content and solute concentration of the solution to be absorbed.

Carbohydrates have been shown to have a marked effect upon gastric secretion (2), motility (3) and evacuation (4). Recently a summary of findings regarding factors involved in the disposition of glucose solutions within the gastro-intestinal tract of humans has been presented by Warren, Karr, Hoffman and Abbott (5).

The object of this investigation has been to determine *a*, the influence of the size of the meal on the emptying rate of the stomach after the intragastric administration of concentrated glucose solutions, and *b*, the alterations in concentration of administered glucose solution by gastric residuum and secretion, and by duodenal secretion and absorption.

EXPERIMENTAL. The rats were anesthetized by injections of 60 mgm. of pento-barbital per kilogram body weight. Pento-barbital was selected because of its comparatively slight effect upon gastric motility (6). As soon as anesthesia was complete (45 min. later), the animals were tied on animal boards and an incision made through the abdominal wall starting at the xiphoid process of the sternum and continuing about an inch caudad. The duodenum was traced for approximately an inch along its course, and at this point a cannula 9 cm. long was inserted with its tip 0.5 inch from the pylorus. The length of the cannula was such that a spinal puncture needle when inserted to the hilt would just reach the orifice at the small end. By attaching a 1 cc. tuberculin syringe to the needle, the fluid could be completely removed from the cannula.

With the cannula in place, the 50 per cent glucose solution was injected slowly and under a constant head of pressure into the stomach through a stom-

¹ Part of the data in this paper is taken from a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Vital Economics of the University of Rochester, June 1938.

ach tube. The fluid introduced distended the stomach somewhat but did not make it full or tense. Since Van Liere and his associates (7) found that the size of the meal affected the evacuation time, we have used two different quantities of 50 per cent glucose in this study.

Fluid appearing in the cannula after the introduction of glucose was removed, measured and expressed into a 100 cc. volumetric flask, the samples for each 15 minute period being pooled and analyzed for sugar by the Bertrand method. At the end of an hour, 0.5 cc. of the fluid in the stomach was withdrawn with a syringe and analyzed so that the concentration of sugar in the gastric contents could be found. The esophagus was ligated near the cardiac sphincter prior to excision of the stomach and that part of the duodenum attached to the cannula. The contents were recovered by slitting the stomach, washing all parts thoroughly and transferring the washings to a volumetric flask for subsequent analysis.

TABLE 1
Coefficients of absorption with intact animals
Milligrams per 100 grams body weight per hour

	1 HOUR	2 HOURS	3 HOURS
Normal*.....	213	181	160
Anesthetized (large meal).....	175	131	119

* Feyder and Pierce, J. Nutrition 9: 435, 1935.

Since we planned to use pento-barbital anesthesia in the experiments described above, and knew that it decreased gastric motility, we wondered whether the extent of the effect of the anesthetic on motility could be estimated from a study of glucose absorption in normal and anesthetized animals. Therefore, rats were given the same dose of pento-barbital we were planning to use in the gastric emptying studies and glucose absorption determined.

RESULTS. The effect of pento-barbital anesthesia upon glucose absorption in intact animals is shown in table 1.

The chart shows curves plotted from data obtained with 26 rats each fed approximately 1000 mgm. of glucose and curves from results with 24 rats each fed approximately 650 mgm. glucose, in the form of a 50 per cent solution. In all experiments as time progressed there was a marked decrease in the rate at which sugar and fluid were discharged from the stomach and changes were more rapid early than late in the experiments. With the smaller meal the concentration of glucose was reduced to about 66 per cent of that with the larger meal during the first 15 minutes and continued to maintain this relative level to the end of the hour. The volume discharged, however, was at first only 80 per cent of that following the larger meal and thereafter nearly the same at each interval; consequently less glucose was emptied throughout from the stomach with the smaller than with the larger meal.

The data in table 2 present findings after the elapse of 1 hour. With the

larger meal all values were higher than those obtained with the smaller meal. The absorption coefficients were calculated on the basis that all of the glucose emptied during the one hour period was absorbed. The value found (series I) agrees closely with that obtained with anesthetized intact animals fed a like amount of glucose (see table 1).

DISCUSSION. The average gastric residuum of 0.4 cc. reduced the concentration of the 50 per cent glucose administered to approximately 40 per cent in both series. When the large meal was fed, a more concentrated solution was emptied from the stomach during the first 15 minutes than with the small meal. Our data at 60 minutes conform more closely to those of Johnston and Ravdin (8) and Ravdin et al. (9) than to those of Karr et al. (10). The difference in results may be due to different techniques.

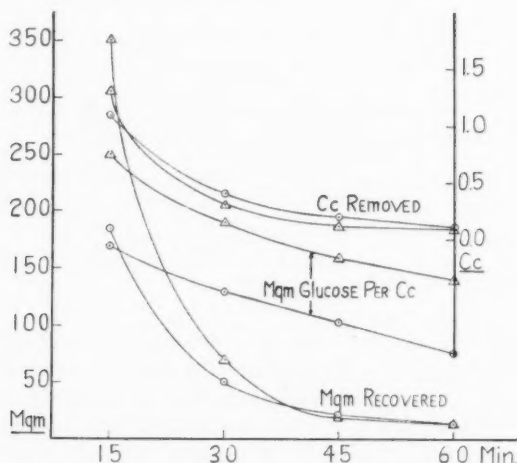


Figure 1 curves show fluid emptied, concentration of glucose in fluid and amount of glucose discharged after feeding 1000 and 650 mgm. glucose respectively.

The secretion added following the two meals was about the same. Our data suggest that there is a limit to the secretory capacity of the stomach. During one hour the glucose concentration in the stomach had fallen to 21.6 and 20.2 per cent, while the average concentrations of the fluid recovered from the cannula were 14.2 and 7.6 per cent respectively. This shows that either fluid secretion or glucose absorption or both is taking place in the part of the duodenum exposed. The amount of residuum found in the stomachs of rats was not constant, and we know that this also holds true for man. This raises the question, what effect does initial gastric dilution of orally administered glucose have upon sugar tolerance curves. Variations in dilution by gastric residuum would tend to alter the inhibitory effects of strong glucose solutions upon gastric motility and emptying.

The fact that 10 and 7.5 per cent of the sugar fed was absorbed in the small portion of exposed duodenum reemphasizes the importance of the duodenum in the absorption of soluble foodstuffs. However, our results point also to the stomach as an important diluting organ in contrast with the findings of Shay et al. (11). Measurements of hydrogen ion concentration of the stomach contents of rats fed 50 per cent glucose indicate that the secretion of the stomach is not normal acidic gastric juice.

Theoretical absorption coefficients were calculated and found to be 180.3 with the large meal and 146.3 with the small. This indicates the close dependence of absorption upon the emptying rate of the stomach. The early rapid outflow from the stomach followed in 5 to 10 minutes by a retardation of flow, agrees well with the observations of Quigley and Phelps (3). Pento-barbital inhibits gastric motility (6) and it is possible that the effect of sugar and anesthesia is additive. If, on the other hand, glucose exerts its action due to contact with duodenal mucosa, the anesthetic by reducing gastric motility may

TABLE 2
Summary of emptying rate at end of 1 hour

	SERIES I	SERIES II
Glucose fed.....	1003.0 mgm.	647.0 mgm.
Glucose discharged.....	458.0 mgm.	269.0 mgm.
Glucose remaining in stomach.....	462.0 mgm.	314.0 mgm.
Glucose concentration in stomach.....	21.6 %	20.2 %
Volume injected.....	1.9 cc.	1.4 cc.
Volume discharged.....	1.9 cc.	1.8 cc.
Volume remaining in stomach.....	2.2 cc.	1.6 cc.
Amount fluid added by secretion.....	2.2 cc.	2.0 cc.
Absorption coefficient (calculated).....	180.3	146.3

decrease the emptying rate, which in turn permits greater dilution within the stomach and so reduces the effect of the concentrated glucose solution on the duodenum.

In a series of intact animals with and without anesthesia the amount of glucose remaining in the stomach was determined 15, 30, 45 and 60 minutes after feeding glucose. All results showed more glucose remaining in the stomach of anesthetized than of non-anesthetized animals at each time interval. The glucose recovered at 15 minutes was 648 vs. 524 mgm., at 30 minutes 579 vs. 530 mgm., at 45 minutes 560 vs. 493 mgm. and at 60 minutes 545 vs. 453 mgm. The data show that pento-barbital retarded gastric emptying and decreased absorption. There is close agreement between the calculated absorption coefficient (table 2) and that actually determined with intact anesthetized animals—180.3 vs. 175. Similar agreement was found when the smaller meal was fed. The data (table 1), as with normal animals, show that absorption decreases with time.

SUMMARY AND CONCLUSIONS

1. Fifty per cent glucose solutions introduced into the stomach are diluted rapidly by residuum and fluid secreted by the stomach. Maximum dilution occurs during the first fifteen minutes.

2. The stomach is important as a diluting mechanism, but in our experiments dilution also occurs in the duodenum.

3. The concentration of glucose in the contents expressed from stomach is lower with a small than with a large meal.

4. The average concentration of glucose remaining in the stomach at the end of one hour is approximately 20 per cent regardless of whether 650 or 1000 mgm. of glucose are introduced in form of 50 per cent solution.

5. The rate of gastric discharge decreases with time. This rate is rapid in the first 15 minutes after feeding, then decreases, attaining a fairly constant state after 45 minutes.

6. Pento-barbital anesthesia reduces rate of gastric discharge and glucose absorption from the intestine.

7. The amount of glucose absorbed is dependent upon the size of the meal fed.

8. Glucose absorption, especially in the first hour, is dependent upon the amount of sugar present in the gastro-intestinal tract.

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SMOOTH MUSCLE MOTOR-UNITS IN SMALL BLOOD VESSELS

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Application of the concept of the motor-unit to smooth muscle has been denied by Rosenblueth and Rioch (1933), on the basis of indirect evidence. Using the cat's nictitating membrane with partially severed nerve supply, they progressively increased the frequency of stimulation of the cervical sympathetic nerve and obtained a corresponding increase in the height and tension of the contraction until it approximated that of the nictitating membrane with an intact nerve supply. Consequently, they considered that the ratio of the response from a preparation with an intact nerve supply to that with a partial supply was a function of the frequency of stimulation, and not a function of the number of fibers cut, as in skeletal muscle, where the existence of a motor-unit is accepted, and the all-or-none principle applies. Rosenblueth and Rioch explained the augmented response on the basis of an increased quantity of a neurohumor and its diffusion to adjacent muscle cells. They concluded that the all-or-none principle and the concept of the motor-unit did not apply to smooth muscle.

In Rosenblueth's laboratory Klopp (1940) reinvestigated the possibility of smooth muscle motor-units in the chronically denervated nictitating membrane. Some of the elements were sensitized by partial denervation. Although the number of smooth muscle cells controlled by a nerve fiber varied with the frequency of stimulation, the responding area was relatively small with a low frequency. Consequently he admitted that a relative division into units is defensible.

From an analysis of the electrical and mechanical records of a similar preparation, Eccles and Magladery (1937) concluded that smooth muscle cells, "units," are all-or-none, although these are not arranged in discrete motor-units.

Bozler (1938) obtained an all-or-none contraction of uterine strips from the cat and guinea pig, in preparations in which all nervous conduction was blocked with cocaine. He explained this conducted contraction on the basis of a muscle syncytium. According to this concept the entire uterus might be considered a single motor-unit. Bozler (1939) suggested that there are two kinds of smooth muscle, namely, visceral muscle, such as that of the uterus and ureter, and muscle with a motor innervation, such as that of the nictitating membrane and the blood vessels. In 1938 he also stated that it seemed unlikely that widespread syncytial connections occurred in the smooth muscle which is supplied by true motor nerves, like that of the blood vessels and the nictitating membrane.

In a preliminary report on the neuro-motor mechanism of small blood vessels in the retrolingual membrane of the frog, Fulton and Lutz (1940) suggested that the smooth muscle of the blood vessels was organized in motor-units. This concept has been supported by further investigation, and evidence has been obtained for syncytial smooth muscle segments in small blood vessels.

METHOD. The retrolingual membrane of the frog, *Rana pipiens*, with brain and medulla destroyed, was exposed for transillumination using the method described by Pratt and Reid (1930). The animal was placed in a Petri dish and the tongue was everted over a glass block, cemented to the bottom of the dish. Sufficient Ringer's solution was added to cover the block and the overlying retrolingual membrane. The entire preparation was placed on the stage of the microscope and illuminated by transmitted light.

A unipolar, silver-glass micro-electrode, one to five microns in diameter at the tip, was made by drawing a glass capillary, containing silver wire, and placed in the field under the microscope by means of an Emerson micromanipulator. For use in stimulation, brief currents from an induction coil (Harvard Apparatus Company) were passed through the micro-electrode. A light-splitting prism and a motion picture camera were used to record significant responses of the small blood vessels to stimulation of minute nerves in the field of the microscope. Experiments were performed on untreated, and cocaineized preparations (1 per cent cocaine hydrochloride).

RESULTS. Brief faradic stimulation of small nerves in the field of the microscope generally produced a diphasic response, dilatation followed by constriction (fig. 1). In each case, the reacting vessels, including arterioles, precapillaries, and muscular capillary origins, constituted a limited vascular pattern, which was only a small portion of the total vascular area of the membrane. Certain capillary origins, possessing modified smooth muscle cells, responded in a sphincter-like manner, independently of the supplying vessel. Except for their muscular origins, the capillaries did not respond, either to nerve stimulation or direct stimulation of the wall. In preparations stained vitally with methylene blue the perivascular nerve plexus appeared to be anatomically continuous. Because stimulation of small nerves in the field of the microscope produced responses which were confined to restricted vascular patterns (fig. 2), the plexus must be considered physiologically discontinuous. The limited responses suggest the concept of a smooth muscle motor-unit.

When the response was diphasic, the area constricted was frequently only a portion of that originally dilated (fig. 1, III). Occasionally nerves were found which produced only one kind of response, either constriction or dilatation, to all strengths of stimulation. These observations imply that separately innervated constrictor and dilator units may be involved. Stimulation of any one of several small nerves produced a response confined to the same local vascular pattern. Such observations imply that axon reflexes were operating in efferent neurones, and are direct evidence for the concept of the smooth muscle motor-unit.

In cocaineized preparations of the retrolingual membrane, stimulation of the

blood vessel wall produced constriction of exactly the same region which responded to nerve stimulation before treatment. Cocaine made the nerve plexus non-functional, since stimulation of small vasomotor nerves produced no response. Consequently, the limited conducted response implies a non-nervous conducting mechanism, such as a muscle syncytium, discontinuous at the junctions of certain vessels. McGill (1909) obtained evidence for a partial syncytium of vascular smooth muscle in the mammal. Our investigation shows that the musculature of the blood vessels of the frog's retrolingual membrane is composed of a large number of discrete smooth muscle units. It does not permit a conclusion concerning the possibility of an all-or-none response of



Fig. 1. I, II, and III are made from enlargements of single frames selected from a motion picture sequence. A, arteriole; B, precapillary; C, capillary; D, nerve; E, micro-electrode. Original magnification, $\times 100$. I. Condition 0.8 sec. before stimulation. II. Condition 8.5 sec. following stimulation. Duration of stimulation 0.8 sec. Latent period 2.5 sec. (from the beginning of stimulation). Bubble at tip of microelectrode indicates stimulation. Dilatation of the arteriole, precapillary, and capillary origin. III. Condition 7.7 seconds later. Constriction, confined to the precapillary and capillary origin.



Fig. 2. I and II are made from enlargements of single frames selected from a motion picture sequence. A, arteriole; B, precapillary; C, capillary; D, capillary; E, nerve; F, micro-electrode. Original magnification, $\times 200$. I. Condition 0.8 sec. before stimulation. II. Condition 4.3 sec. following stimulation. Duration of stimulation 0.8 sec. Latent period 1.5 sec. (from the beginning of stimulation). Bubble at tip of micro-electrode indicates stimulation. Constriction, confined to the precapillary.

vascular smooth muscle, acting either as units or as individual fibers. However, vascular and uterine smooth muscle may be alike in that both appear to be syncytial. Extensive contraction of blood vessels, when a nerve is stimulated, may depend upon the activation of a large number of independent motor-units coordinated through the nerve supply.

In the blood vessels of the retrolingual membrane sufficient nervous tissue is present, in the form of a copious perivascular plexus, to innervate all the contractile elements. As pointed out clearly by Eccles and Magladery (1937) the concept of a sparse innervation of all smooth muscle is no longer tenable. Because of the profuse nerve supply, as well as the syncytium, it is unnecessary to assume the diffusion of a chemical mediator from "key cells" to account

for conducted responses. Obviously, the existence of an abundant innervation does not preclude direct neurohumoral transmission at the nerve-muscle junction, but renders it plausible, since it would be difficult to explain the rapidly conducted, restricted responses of small blood vessels on the basis of diffusion of a chemical mediator from scattered key cells. The abundant innervation also affords a possible mechanism for electrical transmission. The blood vessels comprising a single reacting smooth muscle motor-unit do not influence adjacent and sometimes overlying vessels of another unit. If diffusion occurs from key cells, temporal and spatial factors should determine the progress and extent of the response. We have seen no such evidence for diffusion.

SUMMARY

Stimulation of minute nerves, with a micro-electrode, produced spatially limited vascular responses, generally dilatation followed by constriction, in the small blood vessels of the retrolingual membrane of the frog, *Rana pipiens*. The limited responses suggest the concept of smooth muscle motor-units in small blood vessels.

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CONTRASTING EFFECTS OF LOCAL APPLICATION OF ADRENALIN ON THE DENERVATED IRIS OF THE CAT AND THE MONKEY¹

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In previous communications we pointed out that autonomic responses of the monkey differ from those of the cat (Bender, 1939; Weinstein and Bender, 1941). In the present paper, we wish to report another difference between these two species, namely, in the response of the denervated iris to local application of epinephrine. Observations on the local instillation of adrenalin in the conjunctival sac have previously been made on cats, rabbits, rats, and birds (Drake et al., 1939; Koppanyi, 1926; Meltzer and Meltzer, 1904). The effect on the monkey iris has not been previously compared.

METHOD. Sixty cats and thirty-two monkeys (*macaca mulatta*) were used. Observations were made of the iris of the unanesthetized animal in 1, the normal; 2, sympathetically denervated (s.d.) in which the superior cervical ganglion (s.c.g.) was removed; 3, the completely denervated (c.d.) in which all the ciliary nerves were sectioned behind the globe; 4, the trigeminally denervated in which the fifth nerve was cut intracranially or the ophthalmic division was sectioned, and 5, oculomotor denervated in which the third nerve was cut intracranially. All procedures were performed under aseptic technique.

The pupillary diameter was measured with a millimeter scale under constant illumination. The data in some cats and monkeys were confirmed by controlled cinematographic studies (Lowenstein, 1927). The pupillary images were photographed in the dark on infra red sensitive films. After the films were developed, they were projected on a screen and the pupillary diameter measured.

The adrenalin was instilled into the conjunctival sac in a dose of one drop of 0.1 per cent solution repeated three times every five minutes. In some instances, 1.0 per cent solutions were employed. Subconjunctival injections of drugs were made in 0.1 cc. volume. Many of the instillation and all subconjunctival injection experiments were carried out under nembutal anesthesia in order to avoid the possible secretion of adrenalin because of fright or struggle. The results obtained were the same as in the unanesthetized animal.

RESULTS. I. Adrenalin. (a) *Cat.* The instillation of 0.1 per cent adrenalin in the conjunctival sac of the normal or sympathetically denervated eye of the cat did not result in mydriasis. Blanching due to local vasoconstriction of the

¹ This work has been aided by grants from the Josiah Macy Jr. Foundation and the Dazian Foundation for Medical Research.

palpebral and bulbar conjunctivae was noted. An increase in the number of drops or concentration of adrenalin up to 1.0 per cent was without significant effect, nor did removal of the nictitating membrane alter the results. Preliminary instillation of cocaine did not potentiate the mydriatic effect of local adrenalin. Several authors (Drake et al., 1939; Schlossberg, 1932) have stated that following excision of the s.c.g. in the cat, the ipsilateral pupil dilates after adrenalin is placed in the conjunctival sac. This was never observed in our experiments, even when checked with cinematographic recording under constant illumination. Furthermore subconjunctival injections of 0.1 cc. of 0.1 per cent adrenalin failed to produce any mydriasis in these preparations. The only obvious effects were constriction of the conjunctival vessels and resultant blanching of the membranes, cooling of the ipsilateral ear and widening of the palpebral fissure.

When, however, the iris was completely denervated by sectioning all the ciliary nerves, instillation of one drop of 0.1 per cent adrenalin in the conjunctival sac invariably produced within 4 minutes an increase in from 2 to 4 mm. in the pupillary diameter. Severance of the ophthalmic division of the fifth nerve also produced a sensitivity to local adrenalin. This procedure not only interrupted the trigeminal innervation, but sectioned the post-ganglionic sympathetic fibers of the internal carotid plexus which enter the ophthalmic division, thus rendering the dilator pupillary fibers of the iris sensitive to adrenalin. Subconjunctival injection of 0.1 cc. of 0.05 per cent adrenalin in the eye with the completely denervated iris resulted in a mydriasis within 3 to 4 minutes. Here there was little blanching of the conjunctivae apparently indicating only slight vasoconstriction.

(b) *Monkey*. The same quantity of adrenalin (3 drops of 0.1 per cent solution) instilled into the conjunctival sac of the monkey had no effect on the normal, but dilated the pupil of the s.d. and c.d. iris. Subconjunctival injections of 0.1 cc. of 0.1 per cent epinephrine into the tested eye produced the same effects as local instillation. The mydriatic effect of local adrenalin on the s.d. iris was more conspicuous than that on the c.d. iris. This is in marked contrast to the finding in the cat. Local epinephrine also dilated the pupil of the iris to which the trigeminal nerve supply was interrupted by section of the ophthalmic division. As in the cat, the first division of the fifth cranial nerve conducts the sympathetic fibers to the iris of the monkey. Cutting of the ophthalmic nerve abolishes the mydriasis produced by electric stimulation of the cervical sympathetic trunk and renders the pupillo-dilator fibers sensitive to adrenalin; virtually a post-ganglionic denervation of the sympathetic supply to the iris.

II. Acetylcholine and eserine. Local application of eserine 0.1 per cent constricted the pupil of the normal and that of the oculomotor-denervated iris in the cat and monkey, more rapidly in the latter. The effects of local acetylcholine and eserine on the denervated iris were as observed in the cat by Shen and Cannon (1936). From 3 to 6 days following complete denervation of the iris, there was constriction of the pupil following the application of 0.1 per cent eserine. As the nerve endings went on to complete degeneration, eserine became less effective until no miosis could be elicited. At this stage, however, the addition of one

drop of acetylcholine 0.1 per cent in the eserinated eye produced a rapid miosis. It was during this 2 to 3 week period, when local eserine was without constrictor effect on the c.d. iris, that the acetylcholine sensitization phenomena was greatest. With regeneration of the ciliary nerve, the constrictor action of eserine reappeared and acetylcholine sensitization diminished. There was no essential difference in these respects between the monkey and the cat.

DISCUSSION. We have previously pointed out the contrasting effects of intravenously injected adrenalin and acetylcholine on the denervated iris of the cat and monkey (Bender and Weinstein, 1940). The reactions to local instillation of adrenalin in the denervated eye also show dissimilarity, not only in the reactions of the two species, but between the s.d. and c.d. iris of the cat. This difference may be due to changes in the factors of local diffusion and absorption. Solutions introduced into the conjunctival sac reach the iris by the diffusion through the cornea and absorption by the conjunctival blood vessels which anastomose with ciliary vessels. When the drug is injected subconjunctivally the route of diffusion through the cornea is eliminated and there remains only vascular absorption.

It is of interest that while adrenalin is poorly diffused, eserine is readily taken up from the cat's conjunctival sac. Adrenalin is a vasoconstrictor and produces blanching of the conjunctivae while eserine dilates blood vessels. Observation on the effects of subconjunctival injections makes possible the assumption that vasoconstriction reduces the vascular bed to an extent sufficient to prevent absorption of adrenalin by means of the local circulation. Accordingly, there is no mydriasis obtained. The constriction of the denervated conjunctival vessels by adrenalin in the cat must be more intense than in the monkey, for in the latter there is apparently enough adrenalin absorbed to produce mydriasis. In the iris of the cat in which all the nerves behind the eyeball are severed, subconjunctival adrenalin does not produce complete blanching and the pupil of the denervated iris becomes dilated, again indicating that when vasoconstriction is incomplete, there is better absorption of the drug.

In addition to differences in the absorption mechanism by the conjunctival blood vessels, there is a probability that the corneal permeability plays an important role in altering the pupillary diameter following local instillation of adrenalin. As a matter of fact, the normal corneal epithelium has a selective permeability to various drugs. When its innervation is intact, there is apparently insufficient diffusion of adrenalin from the cat's conjunctival sac to act upon the s.d. iris, even though the latter may be very sensitive to the action of intravenously administered adrenalin. When the fifth nerve or the ciliary nerves are sectioned as in the c.d. iris preparation, the normal metabolism and permeability of the corneal epithelium is altered and thus adrenalin is allowed to diffuse through and act upon the iris. A similar observation was made by Poos (1927) who found that partial denudation of the cornea increased its diffusibility and enabled local adrenalin to dilate the pupil.

In considering the difference in response between the s.d. iris of the cat and monkey, aside from a probable species variation in permeability, it would seem that the denervated conjunctival vessels of the cat are extremely sensitive to

the vasoconstrictor effects of adrenalin while the vascular bed of the monkey is not as markedly constricted by epinephrine and is thus able to absorb a greater quantity of the drug. Once the adrenalin enters the circulation it reaches the iris to act on the dilator fibers. Previous studies have shown that the denervated pupillodilator fibers of the cat are much more reactive than those of the monkey (see table). Thus it is again apparent that the organs deprived of their sympathetic nerve supply are much more sensitive to the humoral effect of adrenalin in the cat than in the monkey.

TABLE 1

Comparative effects of adrenalin on the iris, of the cat and monkey, deprived of its post ganglionic sympathetic nerve supply by the intravenous and conjunctival routes

ADRENALIN	CAT	MONKEY
Intravenous	Marked mydriasis with minute doses 1×10^{-7}	Slight mydriasis even with doses 1×10^{-5}
Subconjunctival	No mydriasis	Moderate mydriasis
Local	No mydriasis	Moderate mydriasis

SUMMARY

1. The normal pupil of both the cat and monkey is unaffected by local application of 0.1 per cent adrenalin.
2. Adrenalin of the same concentration introduced into the conjunctival sac or injected subconjunctivally dilates the sympathetically denervated iris of the monkey but not of the cat.
3. In the completely denervated iris, local adrenalin produces a marked mydriasis which is more conspicuous in the cat than in the monkey.
4. These variations may be due to differences in the mechanism of absorption by the conjunctival blood vessels and the selective permeability of the cornea in each of the species. It is inferred that the conjunctival vessels of the cat constrict much more readily with adrenalin than the same vessels of the monkey.

We are indebted to Dr. Otto Lowenstein of New York University who made the cinematographic records in some of our experiments.

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CHANGES IN THE BALANCE OF RESPIRATORY DRIVES RESULTING FROM OPEN PNEUMOTHORAX¹

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Modification of respiratory rhythm during inflation and deflation of the lungs (Hering and Breuer, 1868; Gad, 1880; Head, 1889, and others) gave early indication of the participation of proprioceptive reflexes in the control of breathing for it was noted that an artificially sustained super-volume of the lungs held breathing in the expiratory position whereas collapse of the lungs resulting from pneumothorax tended to hold respiration in the inspiratory phase (Head). In our observations on pneumothorax under conditions of moderately deep morphine-urethane anesthesia and of intensified asphyxia we have found evidence of a systematic progression of changes in several respiratory drives and a tentative explanation of their causes.

METHOD. The procedures which we employed are similar to those previously described in more detail (Gesell and Moyer, 1935). Dogs, anesthetized with morphine and urethane, were connected with a bank of rebreathing tanks to facilitate the administration of gaseous mixtures of various compositions. A specially constructed pneumothorax cannula, provided with a retaining flange, was sewed air-tight into one side of the chest after rupturing the mediastinal pleurae. Bilateral pneumothorax was thus quickly producible by removing a large stopper at the end of the cannula and normal conditions were as conveniently and rapidly reestablished by returning the stopper and withdrawing the pleural air through a side tube of the cannula. Respiratory movements were followed by recording the circumference changes of the torso. This was done with specially adapted bands, slipping easily over a depilated and powdered skin. Spirometer tracings revealed the actual changes in lung volume (see fig. 4). Downstrokes in each tracing correspond with inspiration and upstroke with expiration.

RESULTS. The changes in breathing illustrated in figure 1 are the result of a suddenly produced pneumothorax while the animal was breathing room air. The collapse of the lungs is complete. The findings are comparable to those of Head on the rabbit in that anesthesia in this particular instance was relatively light. The first inspiration during pneumothorax was markedly intensified and prolonged, giving way eventually to short and weak interruption from the expiratory side of the respiratory center (decreased torso circumference). Since the prolongation of inspiration is greater than the shortening of expiration

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breathing is retarded. As pneumothorax continues, the chemical stimulation of breathing must mount precipitously to high values. Nevertheless inspiration increases in relatively small proportion as compared to the initial increase produced by pneumothorax (see the progressively increasing torsal circumference at the end of inspiration). It is, therefore, concluded that the proprioceptive inspiratory drive originating in the collapse of the lungs must be extremely powerful. Though the intensity of inspiration increases slightly as pneumothorax continues, the duration on the contrary shows a progressive diminution. This is indicative of a development of a counter force (*i.e.*, expiratory activity) tending to produce an earlier inspiratory interruption. Direct evidence supporting this interpretation is seen in the increasing intensity and duration of expiratory activity. (Note the progressively decreasing torsal circumference at the end of the expiratory phase and the increasing duration of expiration running parallel

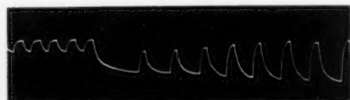


Fig. 1. Respiratory band tracing before and during bilateral open pneumothorax. Inspiration downstroke.

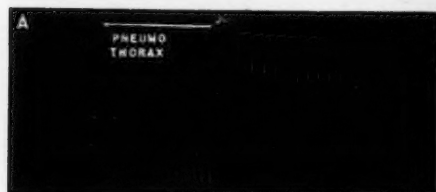


Fig. 2

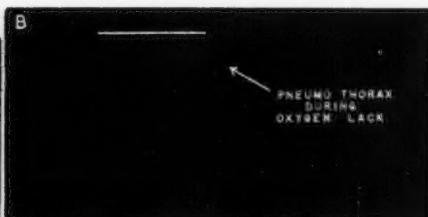


Fig. 3

Figs. 2 and 3. Respiratory band tracing before, during and after pneumothorax. Figure 2, pneumothorax during the administration of room air. Figure 3, pneumothorax during the administration of a gaseous mixture low in oxygen.

with increasing activity.) The equalization of inspiratory and expiratory activity resulting from this disproportionate increase of expiratory activity tends to produce a progressive acceleration of breathing (see Gesell and Hamilton, 1941).

Under deeper anesthesia the effects of pneumothorax were modified in varying degrees. Qualitatively the results were comparable to those already described. In many instances they seemed to differ in quantitative aspects only, see figure 2 for example. Here, the initial retardation of breathing so striking in figure 1, is missing. Nevertheless inspirations are both intensified and prolonged. The prolongation of inspiration occurs at the expense of a drastic cut in the so-called expiratory pause, seen during the preceding eupnea. Inspirations, which were normally shorter, are now longer than their succeeding expiratory phases. The increasing torsal circumferences at the end of succeeding inspirations and the

decreasing circumferences at the end of expirations give evidence of increasing inspiratory and expiratory activity with the progress of pneumothorax. The disproportionate increase of expiratory activity over that of inspiration is more striking than in figure 1. As a consequence there is a more rapid balancing of the inspiratory and expiratory components and a more rapid increase in the frequency of respiratory rhythm. The great shortening of the expiratory phase (see eupnea) in itself allows a high frequency of breathing.

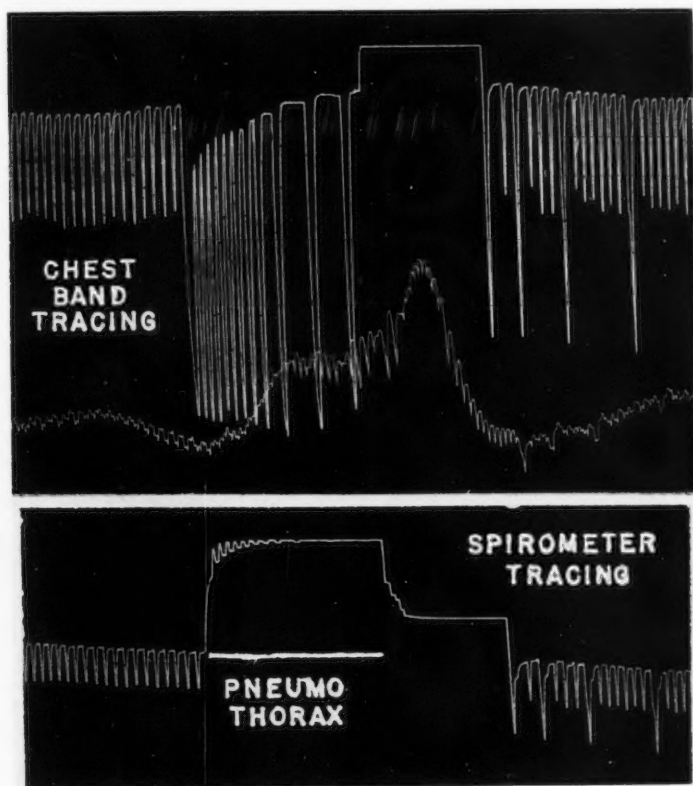


Fig. 4. Chest band and spirometer tracings before, during and after pneumothorax during the administration of room air.

In figure 3 the response to pneumothorax is further altered by the introduction of a new variable. Instead of allowing the animal to breathe room air, as it did five minutes previously in figure 2, an 8 per cent mixture of oxygen in nitrogen was administered and the effects of pneumothorax reobserved. Although the effects of pneumothorax are clearly modified the initial results seen in the first four breaths are qualitatively comparable to those illustrated in figures 2 and 1. There is a prolongation and intensification of inspiration, a progressive increase of both inspiratory and expiratory activity, an equalization of inspiratory and

expiratory components and an accelerated frequency of breathing. Following breath 4, however, there is a progressive retardation of breathing. This new departure in respiratory response can hardly be attributed to a weakening of the respiratory mechanism for the strengths of inspiratory and expiratory contractions are growing. As we shall try to show, the retardation of rhythm seems to be linked with an increasing dominance of expiratory activity.

An accentuation of the results seen in figure 3, in which the preliminary changes of breathing during pneumothorax are virtually eliminated, is not uncommon. In figure 4, for example, only the first inspiration is clearly prolonged. Barring that respiratory cycle, breathing virtually begins at maximum frequency and within a short period of four rapid breaths retardation of breathing is clearly under way. It is significant that expiratory activity is strong in the very first respiratory cycle. The relatively even balance of inspiratory and expiratory activity which is assured by this initially powerful expiratory component would seem to be a logical explanation of the initial high frequency of breathing. As expiratory activity continues to grow in strength, it gains predominance in the respiratory act and holds inspiration more and more in abeyance. The evidence for supereupneic expiratory activity during pneumothorax is obvious in the constricted condition of the torso during the so called "expiratory pause." By extending the eupneic expiratory level, it will be seen that the expiratory circumference is subeupneic and if further allowance be made for the absence of the inward elastic pull of the lungs which normally assists the expiratory muscles it may be assumed that the torsal record under-indicates the power of the expiratory contraction. That is a point worthy of use in the evaluation of all of our results.

DISCUSSION. In the interpretation of our observations the respiratory center is pictured as two half-centers working in reciprocal coordination. By virtue of reciprocating collaterals one half-center alternately dominates the other, for increasing activity in one tends automatically to inhibit the activity in the other. Under usual conditions the driving forces (chemical, proprioceptive and others) acting on both half-centers are adjusted to assure a well balanced respiratory act of suitable rhythm (for details see Gesell, 1940a, 1940b). Should the balance of the respiratory drives be upset to the advantage of either, the expiratory or inspiratory half-center, as it is during artificial superinflation and deflation of the lungs, a respiratory imbalance occurs. Changes in the chemical and proprioceptive drives set in and beget a continuing change in the respiratory act.

It is these changes which we wish to consider: *a*, the initial dominance of inspiratory activity; *b*, the equalization of inspiratory and expiratory activity and its accompanying increasing respiratory rhythm, and *c*, the final dominance of expiratory activity and its associated retardation of breathing.

Granting that the pulmonary vagal stretch receptors are predominantly expiratory excitatory (Worzniak and Gesell, 1939; Gesell, 1939; Gesell and Moyer, 1941; and Gesell and Worzniak, 1941) and that the collapse receptors are predominantly inspiratory excitatory (Head, 1889), it is clear that pneumothorax removes a potent proprioceptive expiratory drive and substitutes in its stead a

powerful and continuing proprioceptive inspiratory drive. This change alone should be sufficient to give dominance to the inspiratory half-center, and once dominance is established the inspiratory half-center is open to further inspiratory stimulation wherever it may originate (Gesell and Hamilton, 1941). Since a rising tension and distortion of the Golgi endings in a contracting muscle automatically reinforces the contraction producing this distortion (Worzniak and Gesell, 1939), and since this distortion must be very powerful in the inspiratory muscles during pneumothorax, it follows that the intensified Golgi reflex would support the dominance of the inspiratory act. It is highly probable that other reinforcing reflexes also exist.

But the forces that tend to terminate the inspiratory discharge and thus give precedence to the existing expiratory drives (Gesell, 1940), also demand consideration. With the chest intact there are several factors which may contribute to these forces: 1. The inherent tendency of self limitation of discharge possessed by the inspiratory half-center (this concept is based on a theoretical increasing threshold of excitation rising above the excitatory stimulus). 2. The simultaneous recovery of the expiratory half-center from its previous discharge combined with a concomitant stimulation primarily of proprioceptive origin. 3. Inhibition of the inspiratory half-center, primarily of a reciprocal nature, originating in the opposing expiratory half-center, and 4, an increasing susceptibility of the inspiratory half-center to inhibition during the progress of each inspiratory discharge. The phenomenon of increased susceptibility to inhibition from previous activity was established by Sherrington and Sowton (1940) and Fulton (1938) in spinal reflexes and confirmed by Gesell and Hamilton (1941) in the respiratory center.

In the analysis of breathing during pneumothorax it becomes essential to appreciate that the collapsed condition of the lungs should theoretically modify each of these four factors. The failure of the lungs to expand with each inspiratory discharge eliminates the inspiratory excitatory component of the vagal stretch reflex. Inspiration, therefore, progresses more slowly thus involving factors 1 and 4 (less fatigue). The failure of the lungs to expand also eliminates the powerful expiratory excitatory component of the vagal stretch reflex thus involving factors 2 and 3 operating through the expiratory half-center. The plausibility of these suggestions may be tested in the converse situation where the respiratory center is subjected to excessive vagal stretch receptor drives, both inspiratory and expiratory (Gesell and Moyer, 1941). Under these conditions breathing is often irregular, with deep, powerful and extremely rapid respiratory contractions interchanging with more shallow respiration. The shallowness of breathing must not, however, be used as an indication of weakness of the smaller inspiration for all of the respiratory movements, deep and shallow alike, transpire with exceptional speed. It therefore seems probable that under the peculiar condition of pulmonary inflation even powerful inspirations are highly susceptible to interruption long before they attain a normal depth. Conversely then a slowly developing inspiration during pneumothorax in which the accelerating vagal proprioceptive drive is missing should attain a greater depth

than normal. Furthermore the elimination of the vagal expiratory excitatory reflex during pneumothorax should diminish the reciprocal interrupting action of the expiratory half-center thus allowing inspiration to reach a still greater depth.

The equalization of inspiratory and expiratory activity and the associated increase in respiratory rhythm. The equalization of inspiratory and expiratory activity which occurs during the progress of pneumothorax is the most apparent cause of the increasing frequency of breathing, for equal activity of the competing centers would tend to equalize the periods of the respiratory cycle and thus permit acceleration (Gesell and Hamilton, 1941). Several factors leading to a greater dominance of expiratory activity were mentioned above. In some still unknown way increasing depth of anesthesia seems to promote a relatively greater expiratory activity. Oxygen lack has the same effects. The progressively increasing asphyxia resulting from a continuance of pneumothorax may, therefore, be regarded as a logical explanation of the relative increase of expiratory activity. This may be related to a growing fatigue of a highly overworked inspiratory half-center. But in addition there is the possibility of a complementary proprioceptive reinforcement of expiratory activity which may prove to be the most important factor of all. Proprioceptive sensory endings in the respiratory muscles and their associated tendons and joints must suffer distortion in direct proportion to the intensity of the respiratory contractions. Since the inspiratory contractions are extremely powerful the proprioceptive excitation originating in both the inspiratory or expiratory muscles and tendons could be intense. Adding to these potentialities the universal power of central summation of impinging signals and of central after-discharge, a plausible mechanism of increasing expiratory activity is at hand. Because inspiratory activity is almost maximum and expiratory activity relatively weak at the beginning of pneumothorax a disproportionate increase in expiratory activity is understandable.

The final dominance of expiratory activity and its associated retardation of breathing. If the disproportionate increase of expiratory activity continues, a point is ultimately reached in which expiration dominates the respiratory cycle. The expiratory phase increases in duration and the frequency of breathing diminishes. The conditions are comparable to the graded decrease in respiratory rhythm produced by a graded inflation of the lungs in which expiratory activity is correspondingly increased. Due to the increasing reciprocal inhibition of the inspiratory half-center the discharges of this center are progressively delayed.

The evidence for the existence of a super-expiratory activity at the end of pneumothorax is illustrated in the spirometer record of figure 4. When the lungs are sucked back to the thoracic cage and the opening in the chest occluded, their volume is seen to be considerably smaller than it was at the end of expirations in the preceding eupnea. This indicates that the chest was constricted more than normal. Now when the chest of an intact animal is *passively* compressed (vagi intact), breathing usually accelerates, due to a new balance in the proprioceptive drives. The converse stoppage and retardation of breathing noted in the *actively* constricted chest must have a significance of its own. Everything points to the fact that an excessive expiratory activity already

existing during pneumothorax is continued and augmented long after pneumothorax is abolished. Continuance of pre-existing super-expiratory activity is made possible on reinflation of the lungs by the withdrawal of the powerful inspiratory drive originating in the collapse receptors plus a highly probable intensified expiratory drive originating in the stretch receptors. Due to the prolonged collapse of the lungs, reinflation may produce a relatively strong discharge of the proprioceptive endings even though the lungs be smaller than normal. The reduced chemical stimulation resulting from a refilling of the lungs may also play a part in prolonging the expiratory phase. While an improved ventilation of the blood undoubtedly occurs it is reasonably certain that the chemical stimulation remains higher than normal. This gives unusual interest to the prolonged "apnea." Only as asphyxia increases to still higher values is the dominance of the expiratory half-center ultimately interrupted. But even after breathing has been reinitiated indications of a protracted though diminishing super expiratory activity still exist, namely, a subnormal expiratory lung volume and a subnormal frequency of breathing for the first seven breaths.

SUMMARY AND CONCLUSIONS

Bilateral open pneumothorax in the anesthetized dog produced a systematic progression of changes in breathing. The first effect was a marked intensification and prolongation of inspiratory activity associated with a relatively weak expiratory activity. The second effect was a slowly increasing intensity of both inspiratory and expiratory activity in which the increase of expiratory activity predominated. This led to a shortening of the inspiratory phase and to an acceleration of breathing. Acceleration of breathing continued up to an equalization of inspiratory and expiratory activity. Passing that point expiratory activity predominated, leading to a marked prolongation of the expiratory phase and to a diminution in the respiratory rhythm.

The marked intensification and prolongation of the inspiratory act at the beginning of pneumothorax is attributed to a combined change in vagal proprioceptive drives—a powerful inspiratory excitatory pulmonary deflation reflex replaces a predominantly expiratory excitatory stretch reflex.

The acceleration of the frequency of breathing is attributed to a disproportionately increasing intensity of expiratory activity. This increasing expiratory activity is thought to originate in the increasing asphyxial chemical drive operating more powerfully on the expiratory half-center and in a progressive synaptic summation of expiratory drives originating in excessive deformation or proprioceptive endings in the respiratory muscles and their attachments and joints.

The final slowing of respiratory rhythm is attributed to an intensified reciprocal inhibition of the inspiratory half-center associated with the increasing predominance of expiratory activity.

Without an early development of expiratory activity an animal must die in inspiratory tonus even though the chest wound be relatively small, for without powerful alternating activity of the inspiratory and expiratory muscles tidal air must be inadequate.

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THE EXPERIMENTAL PRODUCTION OF A HEMOPHILIA-LIKE CONDITION IN HEPARINIZED MICE¹

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A vital mechanism exists in the organism which brings about the cessation of bleeding from a wound. Duke in 1910 introduced the bleeding time test which measures the duration of bleeding from a skin wound (1). In spite of the numerous reports on heparin affecting coagulation time, we found no organized study regarding the effect of heparin upon the bleeding time. Only occasionally has the bleeding time been determined in patients who received heparin intravenously (2). The bleeding time in these instances was found to be normal. The problem which remains to be solved is, if any correlation exists between the bleeding time and the coagulative property of the blood.

Since heparin is believed to be a physiological anticoagulant (3), we undertook to test its influence upon the bleeding time. In order to study the bleeding time experimentally, it was necessary to employ a reliable method. Doettl and Ripke (4) described a method in mice which we examined for its accuracy and suitability. We attempted to determine whether any correlation exists between the coagulation time and the bleeding time; if injections of moderate or excessive doses of heparin would influence the bleeding time; and finally, what clinical and pathological changes could be observed after single or repeated injections of heparin.

METHODS. The method which Doettl and Ripke introduced for the determination of bleeding time in mice was modified slightly.⁴ The mouse was placed in a brass tube 7.5 cm. long, a perforated cork was fitted in one side and the tail was inserted in a lucite tail holder which closed the brass cage on the opposite side. Thus the mouse lost its freedom of movement, but was still in a comfortable position. The brass cylinder was suspended at an angle of approximately 40 degrees by a clamp so that the tail was immersed in a physiological saline bath.

The solution was kept at a constant temperature (37.5°C.). The normal saline was changed for each test and in cases of prolonged bleeding whenever the

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³ George A. Breon Fellow in Experimental Medicine.

⁴ Personal communication from the Elberfeld Physiological Institute of the I. G. Farbenindustrie A G, 1940.

blood made the solution cloudy. A stylet with a needle point was employed to cut one of the tail veins which are prominent. Bleeding time was measured from the moment the blood was seen emerging from the wound until the flow of red blood stopped. We differentiated five flows: very strong, strong, medium, feeble and very feeble. Whenever arterial flows were obtained, they were easily distinguished by their pulsating character; these were excluded from normal venous flow values. Normal values were determined in mice by subjecting the animals to repeated tests in order to establish the limits of normal variation, and to ascertain how well the values would check in the same animal. The effect of temperature was also studied in a group of mice to determine whether this would alter the normal bleeding time.

The heparin studies were carried out with four preparations of heparin: two products of the Connaught Laboratories, Toronto, 100 and 110 units per milligram (CLT-100, CLT-110), the commercial product of Hoffmann-LaRoche, 2000 units per cc. (HLR), and the commercial material of Hynson, Westcott and Dunning, 1 milligram preventing coagulation of 7.5 cc. of shed cat's blood for 24 hours (HWD). The potency of these various preparations was checked by coagulation studies on dog's blood prior to their use in mice. We found the comparative potencies of CLT-100 and HWD to be similar to the assay of Charles and Scott (5). One unit HLR was one-fifth as potent as 1 unit CLT-110. Each dose of heparin injected was expressed per 20 grams of mouse weight and this was dissolved in 0.5 or 1.0 cc. of physiological saline.

Three groups of heparinized mice were studied. In one group gradually increasing doses of heparin were injected and the bleeding time was determined 1 to 3 hours afterwards. In the second group the cumulative effect of the three preparations of heparin upon the bleeding time and hematoma formation was studied in 19 test and three control mice. The preparations were injected subcutaneously at 12 hour intervals eleven times. In the last group bleeding time, coagulation time, platelet count and hematoma formation were studied from 1 to 7 hours following heparinization. The coagulation studies were done from tail blood by the capillary method which gave an average value of 114 seconds in 11 normal mice. Platelet counts were done by the method of Vilariño and Pimentel (6) on 0.2 cc. of blood obtained from the mouse's heart. The average platelet count was found to be 277,000 in 13 mice.

Adult male and female mice fed Purina Fox Chow (Purina Mills, St. Louis, Mo.) and crushed oats were used.

RESULTS. Following venepuncture a red flow of blood issued from the wound and flowed directly to the bottom of the container through the normal saline solution in an ever diminishing stream until the flow finally ceased. Movements of the tail disturbed the steady flow of blood and made the observation more difficult. At times we observed an intermittent fluctuation in the strength of blood flow. Occasionally a colorless or a pink flow was observed under strong illumination after the red flow had stopped. We believe this pink or colorless flow is either lymph, tissue fluid, or plasma which may filter around the clot. The clot first forms inside of the wound and often extends to the surface.

A. Normal mice. In order to show that venous flows of different magnitude do occur and that repeated tests on the same animal vary, findings on 25 normal mice are listed in table 1. The determinations were repeated the following day

TABLE 1
Repeated determinations of bleeding time in normal mice (males)

NUMBER	COLOR	WEIGHT IN GRAMS	BLEEDING TIME 10-16-40	BLEEDING TIME 10-17-40	BLEEDING TIME 10-23-40	AVERAGE
			In seconds			
1	W	20	s 60.4 j	s 58.8	s 59.4	59.5
2	W	26	s 69.0	s 66.9	s 113.3	83.1
3	W	24	f 46.4	m 48.2	f 29.0 j	41.2
4	B	22	m 35.4	m 37.8	ss 90.0 A	36.6 +
5	B	28	f 56.0	s 57.6	f 28.6	55.3
					m 79.1 j	
6	W	25	m 27.5	f 19.3	m 53.4	30.3
				f 21.1		
7	W	28	f 23.1	m 33.3	m 35.4	30.6 +
					ss 108.5 A	
8	W	25	f 25.4	f 44.2	m 40.5	36.7
9	W	26	s 41.0 j	f 28.4	m 22.4	30.2
10	B	22	ff 18.7	s 36.8 j	m 45.8 j	33.8
11	W	24	s 56.4	s 71.7	m 57.2	61.8
12	B	18	s 35.6	f 15.4	f 33.8 j	35.5
				s 57.2 j		
13	B	18	s 49.7 j	s 64.2	s 46.6 j	53.5
14	W	19	f 33.8	f 30.2	f 40.2	46.3
			m 53.4 j		s 73.8 j	
15	W	20	s 63.4	f 44.9	s 156.0	88.1
16	W	22	s 72.5	f 41.9 j	f 27.0	59.8
					m 97.8	
17	W	22	m 27.9 j	ss 172.6 jA	s 82.2	55.1 +
18	W	26	m 21.0	s 76.8	m 99.9	65.9
19	W	20	s 115.0 j	m 32.1 j	s 71.0 j	72.7
20	W	24	m 58.5	s 26.6	s 18.4	34.5
21	W	22	s 66.2	f 31.6	m 48.2	48.7
22	W	22	f 19.0	m 66.6 j	s 109.7	60.0
			s 44.7			
23	W	18	f 59.3 j	s 44.2 j	f 31.2	56.5
					s 91.3 j	
24	W	24	m 25.2	m 43.8 j	ss 130.1 A	34.5 +
25	W	20	s 73.4	f 24.4	f 41.7 j	45.0
					m 40.3	

. = very strong, s = strong, m = medium, f = feeble, ff = very feeble; j = movement of tail; A = arterial flow; + = arterial flow not included in average value; W = white, B = black.

and after one week. We believe the variation in bleeding time is probably due to differences in the size of the wound, a condition which is difficult to control even though the same lancet was used every time. It is evident from these re-

sults that a difference of ± 30 seconds, in contradiction to Doettl and Ripke (4), should be regarded well within the normal range. The differentiation in strength of the flow shows that stronger flows usually have longer values, although exceptions frequently occur. Whenever the artery was inadvertently stabbed, the very strong pulsating flow was longer. Arterial values ranged from 90 to 212 seconds in 11 mice; the average was 122 seconds.

In figure 1 the frequency distribution of 310 determinations of venous bleeding time in 118 normal mice is shown. The limits of variation on these mice ranged from 15.4 seconds to 220 seconds; the average was 54 seconds.

In agreement with other investigators (4, 7) we found that lower temperatures are capable of prolonging the bleeding time.

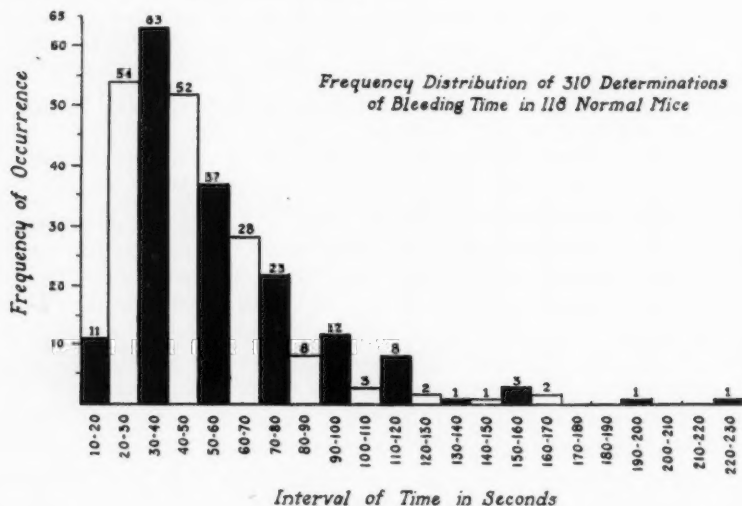


Fig. 1

B. Heparinized mice. Table 2 shows the effect of two preparations of heparin upon the bleeding time of 98 mice. Doses of heparin HLR varying from 1 to 4000 units were injected into 59 mice without any prolongation in bleeding time. In a second series of 39 mice which received from 20 to 800 units CLT-100, three of the animals had bleeding times from 23 to 31 minutes.

Toxicity studies conducted in 10 mice by injecting 3000 to 4000 units of HLR, 800 units of CLT-100 and 1000 units of CLT-110 showed that neither preparation was toxic. The animals which died following injection of massive doses of heparin either developed extensive hematomas on the back due to trauma of injection, or died following the bleeding time test after cleaning their tails. This was never observed in normal mice.

Since the bleeding time was prolonged in some of the mice only after massive doses of heparin, we decided to make simultaneous studies of the effect of heparin on the bleeding time, coagulation time, and the platelet count. For this purpose

a more potent preparation of heparin, CLT-100, was injected into 28 mice. The results upon 16 of these mice are shown in table 3.

Nine mice which received from 5 to 20 units were studied from 47 to 106 minutes following the injection. These animals had normal bleeding times while their coagulation times were somewhat prolonged. The data suggested that longer time intervals were required following subcutaneous injections of heparin to prolong the coagulation time; we therefore studied the remaining

TABLE 2

Effect on bleeding time of two preparations of heparin in various units injected into mice

NUMBER OF MICE	NUMBER OF DETERMINATIONS	PREPARATION	UNITS* ADMINISTERED PER 20 GRAM MOUSE WEIGHT	ROUTE OF INJECTION	AVERAGE BLEEDING TIME
17	20	HLR	1-20	I.P.	1' 3.8"
6	7	HLR	10-20	S.C.	1' 16.6"
16	21	HLR	50-100	S.C.	51.2"
5	8	HLR	200	S.C.	35.3"
4	5	HLR	500	S.C.	59.5"
4	6	HLR	1000	S.C.	45.1"
1	2	HLR	2000	S.C.	55.4"
4	6	HLR	3000	S.C.	1' 23.7"
1	2	HLR	3000	I.P.	3' 52.0"
1	1	HLR	4000	S.C.	1' 13.6"
4	9	CLT-100	20	S.C.	54.2"
8	11	CLT-100	50	S.C.	39.5"
6	8	CLT-100	100	S.C.	52.5"
3	4	CLT-100	150	S.C.	1' 55.9"
1	1	CLT-100	150	S.C.	5' 17.8"
8	12	CLT-100	200	S.C.	52.8"
1	2	CLT-100	400	S.C.	53.2"
1	1	CLT-100	400	S.C.	30' 48.0"
1	1	CLT-100	400	S.C.	27' 58.0"
2	2	CLT-100	800	S.C.	1' 33.9"
1	1	CLT-100	800	S.C.	23' 53.0"
17	22	Controls with isotonic saline S.C.			1' 22.8"
13	17	Controls five days after subcutaneous injection of 50 to 800 units of heparin CLT-100			56.2"

* CLT-100 found to be five times as potent as HLR; I.P. = intraperitoneally, S.C. = subcutaneously.

animals after longer periods of time. Six mice were injected with 200 units of heparin. The bleeding times were determined from 169 to 263 minutes later. Out of this group, one mouse had a bleeding time of 30 minutes following the fourth prick, another 27 minutes, 40 seconds following the first prick. One of these mice bled to death during the test, the first time that this had occurred. In two animals the bleeding time was slightly prolonged, while in two others it was normal. Six animals received 500 units; the time of heparin action was 1 to 7 hours. Two mice in this group showed an increased bleeding time; one,

58 minutes, 28 seconds following the second prick, another 60 minutes following the second prick. The remaining four mice had normal bleeding times. Seven

TABLE 3

Comparison between bleeding time, platelet count, and coagulation time following subcutaneous injection of heparin (Connaught Laboratories, Toronto, 110 units per milligram) in various doses per 20 grams mouse weight

GROUP VII MOUSE NUMBER SEX	BLEEDING TIME BEFORE INJECTION IN SECONDS	UNITS OF HEPARIN	DURATION OF HEPARIN ACTION UNTIL BLEEDING TIME TEST	BLEEDING TIME AFTER INJECTION	DURATION OF HEPARIN ACTION UNTIL COAGULA- TION TIME TEST	COAGULA- TION TIME	PLATELET COUNT IN THOUSANDS
1 M	33	5	1h 16'	ss 1' 37" A	1h 18'	2' 15"	228
36 M	37	5	3h 30'	f 44"	3h 39'	16'	218
16 F	112	10	1h 30'	f 24"	1h 32'	4' 30"	128
7 M	53	20	1h 28'	ss 1' 31" A	1h 31'	7' 45"	242
45 F	42	200	2h 56'	ss 1' 15"	3h 41'	>6 hours	246
57 F	88	200	4h 10'	s 2' 05" j f 4' 15" j	4h 21'	>6 hours	264
46 F	42	200	4h 23'	ss 27' 14" I	4h 54'	>6 hours	Lost
12 F	66	500	3h 00'	s 1' 30" m 58' 28" I	—	—	380
17 F	71	500	5h 40'	s 2' 02"	6h 48'	>6 hours	226
			5h 46'	f 60' 00" j I			
26 F	30	500	6h 53'	s 2' 04" s 2' 12" m 1' 26"	7h 03'	>6 hours	198
25 F	48	500	7h 05'	m 1' 15" f 37" m 1' 05"	7h 14'	>6 hours	182
37 M	45	1000	4h 10'	m 40" m 38" m 1' 01" f 1' 21" f 1' 23"	4h 25'	>6 hours	190
27 F	33	1000	4h 20'	f 30" f 1' 02" f 1' 20" s 10' 26" I	4h 44'	>6 hours	212
28 M	54	1000	4h 34'	m 57" f 59" m 60"	4h 41'	>6 hours	186
13 F	66	1000	4h 55'	m 1' 35" f 44"	—	—	408
18 F	50	1000	7h 12'	s 36' 15" I m 1' 11" m 33' 00" I	8h 00'	>6 hours	218

M = male, F = female; ss = very strong, s = strong, m = medium, f = feeble; I = intermittent fluctuation in strength of flow; j = movement of tail; A = arterial flow.

mice received 1000 units. After three and one-half hours to seven hours one of the animals had a bleeding time of 10 minutes, 26 seconds; four had bleeding times over 22 minutes, while only two mice had normal values. The prolonged

bleeding times occurred in two mice after the first prick wound, in the others it followed second, third and fourth prickings. It may be noted that in the two animals which received 1000 units, normal bleeding times occurred even after the fifth and seventh pricking. Coagulation time studies on 15 mice showed values longer than six hours while 2 mice had values of 1.9 and 2.6 hours. The platelet count ranged between 182,000 and 408,000 in 16 mice which had received more than 200 units of heparin. In doses of 200 to 1000 units of CLT-110, heparin produced a prolongation of coagulation time in all of the mice and increased the bleeding time in 9 out of 19 mice.

Pathological studies of this group of 28 mice revealed hematomas in 12 of the animals. The blood of three of these hematomas was in a fluid state which coagulated a few minutes after being placed in test tubes. The hematomas varied in size from 0.5 by 1 cm. to 1 by 4 cm. Gross inspection of the animals showed the mice had pale ears, feet and tails. Prior to necropsy the animals which had hemorrhages were cold, inactive and the fur was roughened. Inspection of the brain, heart, lungs and the abdominal viscera showed these to be extremely pale. The heart was usually contracting; fluid removed from the heart was not as deeply colored as the blood from the hearts of normal mice.

Following repeated injections of three preparations of heparin. Six hours after the fifth and eleventh injections, the bleeding time was normal in all of the nineteen mice. Eight out of nine mice which received 10 to 200 units CLT-100 and four out of six mice which received from 50 to 250 units HLR developed hematomas. None of the four animals which were injected with 1.1 to 2.2 mgm. HWD exhibited any bleeding tendencies.

DISCUSSION. The tendency to bleed was manifest by hemorrhages and hematoma formation at the site of injection, or continued bleeding from the prick wound following a normal bleeding time when the mouse disturbed the clot while cleaning its tail. Animals which received 1000 units of heparin (CLT-110) showed prolonged bleeding times in five out of seven mice. The long bleeding time, in many instances, was demonstrated only after repeated prick wounds in the same area of the tail vein. On the other hand, some mice which received 200 to 1000 units of heparin did not exhibit any prolongation of bleeding time even if stabbed five or more times. These findings are especially significant because normal mice never showed any undue prolongation of the bleeding time after repeated prickings. These observations support the possibility that a mechanism exists in the skin or tissue fluid which inhibits long bleeding and which is apparently exhausted either by repeated prickings or the infliction of a large wound in excessively heparinized mice. The organ from which the hemorrhage occurs may play a rôle, since Tocantins (8) has suggested that the hemostatic power of the skin differs from that of other organs.

The view generally accepted today is that no correlation exists between the coagulation time and the bleeding time. The fact is apparent that in hemophilia, not only the coagulation of blood, but also the ability to stop the flow of blood from a wound is disturbed. In this respect we believe heparinized mice are similar to hemophiliacs, since both show a prolonged coagulation time and a bleeding tendency.

Since heparinized mice exhibit similarities in their pathological anatomy and clinical behavior to hemophiliacs, table 4 has been constructed to compare the essential features of blood in the two conditions. The coagulation time and bleeding time have been discussed already. Erythrocyte, leucocyte and platelet counts are essentially normal (9, 10, 11). The influence of heparin on prothrombin and calcium has not been investigated in vivo; in studies in vitro, however, heparin was found to have no effect on calcium (11) or prothrombin

TABLE 4
Essential features of blood in hemophilia and in heparinized animals

CHARACTERISTICS	HEMOPHILIA	HEPARINIZED ANIMALS
Coagulation time.....	Increased	Increased
Bleeding time.....	Normal or prolonged (20)	Normal (2, *) or prolonged (*)
Platelet count.....	Normal or increased (20)	Normal (9, 10, *), decreased or increased (10, *)
Erythrocyte count.....	Normal (20)	Normal (9, 11**)
Leucocyte count:		
Total.....	Normal or slightly decreased (20)	Normal (9, 11**) or decreased (11**)
Differential.....	Normal (20)	Normal (11**)
Prothrombin.....	Normal (13)	Normal (12***)
Calcium.....	Normal (13, 20)	Normal (11**)
Fibrinogen.....	Normal (13, 20)	Normal ?
Syneresis (retractility of clot).....	Present (15, 20); irregular surface (16)	Present (16); irregular surface (16)
Thixotropy (reclotting phenomenon).....	Present (15, 17)	Present (17)
Resistance of platelets.....	Increased (15)	
Thromboplastin (thrombo-kinase).....	Decreased (14); normal (13)	
Antithrombin.....	Normal (13, 15); increased (23)	
Heparin.....*	Normal (21); increased (24)	
Coagulation factor.....	Decreased (22)	
Unidentified factor in skin or tissue fluid.....		Normal or decreased (*)

* Data given in this paper.

** Blood heparinized in vitro.

*** Plasma heparinized in vitro.

(12). We did not find any reference on the fibrinogen content following heparinization in vivo or in vitro. Prothrombin (13) and fibrinogen (13, 14), however, were found to be normal in hemophilia. Syneresis (clot retraction), which was observed by Minot and Lee (15) in hemophilic blood, is present in the blood of heparinized animals. The retracted clot in heparinized and hemophilic blood is irregular and can be distinguished from a clot of normal blood which exhibits smooth borders (16). The reclotting phenomenon (thixotropy), first described by Minot and Lee (15) in hemophilia, is also present in heparinized blood (17).

Greater stability of platelets of hemophilic plasma was observed by Minot and Lee (15). Whether this phenomenon exists in blood of heparinized animals has not been established.

Table 4 suggests that other factors remain to be discussed regarding their possible rôle in hemophilia. It is known that heparin and thromboplastin are antagonistic (18). If it is true that heparin is also neutralized by thromboplastin *in vivo*, it may be possible that the prolongation of bleeding time in heparinized mice is due to this reaction. Heparin injected subcutaneously neutralized a factor whose function is to stop bleeding from a wound. This neutralization is dependent on the amount of heparin injected into the animal and the quantity of the unidentified factor present in the animal, since some mice require greater amounts of heparin than others.

We believe that neither the absolute amount of anti-coagulant substance (heparin, antithrombin) or coagulant substance (thromboplastin, coagulation globulin) studied alone suffices to comprehend the pathogenesis of the coagulation disturbance in hemophilia. We propose that normally the coagulative properties of blood are kept in equilibrium by the production of coagulative and anti-coagulant substances in the organism, and that their release into the circulation is regulated by an unknown mechanism. This mechanism is greatly disturbed in hemophilia. It can be postulated that whatever the factor may be which is deficient or lacking in hemophilic blood, it seems to be present in greater quantities in the skin, or tissue fluid. In this connection it has been observed that during or following protracted hemorrhages the coagulation time of hemophilic blood or plasma was observed to be normal (19, 20).

The phenomena of prolonged coagulation time coexisting with a normal bleeding time in the presence of a bleeding tendency in heparinized mice, their clinical behavior, and the observation of the presence of an unidentified factor in the skin which is exhausted in some of the animals may be considered similar to hemophilia. We, therefore, believe that we have succeeded in the experimental production of a hemophilia-like condition in mice.

SUMMARY

1. The method of Doettl and Ripke to determine bleeding time in mice was found reliable.

2. Three hundred and ten venous bleeding time determinations in 118 normal mice ranging between 15.4 to 220 seconds had an average bleeding time of 54 seconds. Unlike Doettl and Ripke, values agreeing within ± 30 seconds on consecutive days were found to be normal. The observation that variations in temperature influence the bleeding time was verified.

3. The effect of four preparations of heparin was studied on the bleeding time. Heparin (Connaught Laboratories, 110 units per milligram) in large doses (1000 units per 20 grams weight, injected subcutaneously) had no toxic effect.

4. There is a definite relationship between the units of heparin injected and the prolongation of clotting time. Such a relation to bleeding time does not exist in smaller doses (5 to 100 units per 20 grams' weight), whereas in excessive

doses (200 to 1000 units per 20 grams weight), and then only in some instances, was there an increase of both the bleeding time and coagulation time.

5. Repeated prickings in some excessively heparinized mice produced a prolonged bleeding time after one or several normal values. This never occurred in normal mice.

6. It is believed that an unidentified factor exists in the skin (or tissue fluid) which may be exhausted by the injection of heparin, resulting in a prolongation of the bleeding time.

7. A correlation between the bleeding time and coagulation time was found in heparinized mice. It is believed that these mice are similar to hemophiliacs because both show a prolonged coagulation time and a bleeding tendency.

8. Essential similarities of the blood, pathological anatomy and clinical behavior of hemophiliacs and heparinized animals are discussed.

9. An equilibrium between coagulant and anti-coagulant substances is believed to be present in normal circulating blood. This is greatly disturbed in hemophilia, resulting in a relative increase of anti-coagulant substance(s) in hemophilic blood.

We wish to thank Dr. A. F. Charles of the Connaught Laboratories, University of Toronto, for his preparation of heparin (100 units per milligram), and the pharmaceutical houses, Hoffmann-LaRoche, Nutley, New Jersey, and Hynson, Westcott and Dunning, Baltimore, for their preparations of heparin.

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THE REGULATION OF ARTERIAL BLOOD PRESSURE IN THE SEAL DURING DIVING

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Many warm blooded animals can dive for 10 minutes or more, and in all those which have been examined the heart slows during a dive. Even non-diving mammals, like man, may show during diving a pronounced bradycardia, which appears to be a common and perhaps universal circulatory change during submersion (Irving, Scholander and Grinnell, 1941a). The bradycardia of the diving seal is the most pronounced yet observed, for the heart beating normally at more than 100 per minute may slow immediately after submersion to a frequency of about 10, which is maintained during 15 or 20 minutes of diving (Scholander, 1940).

With such a bradycardia it may be wondered what is the state of the circulation and particularly the blood pressure. The alertness of the diving seal gives no evidence of inadequacy of the cerebral circulation such as would promptly appear if the heart of a non-diver were slowed to 10 per cent of its usual frequency. During the bradycardia of the diving muskrat it has been stated that the blood pressure may remain high (Koppanyi and Dooley, 1929). In spontaneous pauses of breathing of anesthetized seals the heart may slow below half the normal frequency, but the arterial blood pressure does not diminish (Irving, Solandt, Solandt and Fisher, 1935).

We have observed the arterial blood pressure and various aspects of the circulation during the bradycardia of diving in common harbor seals, *Phoca vitulina*. The animals were young seals which were examined during the summers of 1940 and 1941. One group of seals weighed between 20 and 25 kgm. in 1940, and another group weighed around 35 kgm. when examined in 1941, but their behavior during diving was similar.

METHODS. The seal was fixed by means of padded iron bows to a board with the head and hind flippers slightly elevated. This board was then suspended horizontally in a bathtub so that the body was submerged but the hind flippers and the nostrils were just above water. When properly secured in this fashion, which is described and illustrated by Scholander (1940), the seal would remain quietly for many hours and would even go to sleep. In order to carry on an experimental dive, the head end of the board was tipped below the water surface. In this fashion an experimental dive could be accomplished by a small change of the axis of the animal.

For recording the heart frequency an electrical heart counter devised by S. W. Grinnell (Grinnell, Irving and Scholander, 1941) was used. By means of suitable amplification this instrument applied directly the electrical changes from the heart beat to operate a pen against the tension of a rather stiff spring. The records gave reliable counts of the heart beats, which could be clearly distinguished except during violent muscular activity. Since the record was written directly on paper, continuous records were often made and observed during many hours of experimentation. The electrodes were short steel needles insulated with fine silk and lacquer except for about a centimeter at the point. These electrodes were inserted into the blubber on either side and just behind the front flippers. The electrodes served likewise as leads to the electrocardiograph with which records were taken at critical points in the experiments and in some cases during long periods of experimental dives.

For observing the arterial blood pressure a section of the femoral artery midway along the short femur of the seal was dissected and cannulated. Arterial pressure records were also observed in a small artery about 1 mm. in bore situated between the toes of the hind flipper. For a control as to the practical zero level of blood pressure in the hind flipper, a small vein was likewise cannulated. This vein was subjected to the same small disturbance of level as were the arteries at the time of the experimental dives. It showed in practice that the changes in hydrostatic pressure during experimental diving were not significant.

The cannulae were connected by means of strong-walled rubber tubing to a Harvard membrane manometer which recorded through a lever upon smoked paper. Its inertia proved to be too large to follow the rapid changes in pressure at systole. During the prolonged interval, often lasting 10 seconds or more, between heart beats during diving the record showed the pressure changes in a reliable manner. For such slow changes the movement of the mercury manometer was likewise reliable and it was used for part of the recording in order to give direct records of the absolute level of pressure. The movements of the mercury manometer were recorded upon the moving paper by manual operation of the recording pen to follow the position of the mercury meniscus. The manual recording was as rapid as the movements of the mercury were significant and was not disturbed during the extreme changes in blood pressure which occurred in some of the experiments.

Cardiac action during diving. The heart action of the seals *Cystophora* and *Halichoerus* has been described with electrocardiographic records by Scholander (1940). Portions of a record are shown in figure 1, indicating the frequency of the heart beats of a *Phoca* as registered with Grinnell's electrical heart counter. The record shows the frequencies of the seal's heart before and at the start of a 15-minute dive, in three short periods during the dive, at the time of emergence and after the dive. These records are cut from a continuous record covering the whole period of the dive and recovery. The duration of the interval between the second and third beats in the longer dive, amounting to 18 seconds, is about as long as any which has been recorded, but the record is typical of the operation

of the heart of a seal which is accustomed to experimental dives. In the lower part of figure 1 is shown the complete record of the frequency of the heart during a short dive lasting 33 seconds and indicating the promptness of the development and release of the bradycardia.

In figure 2 are shown electrocardiograms of a seal's heart at the start of a dive, at intervals during the dive and during emergence and recovery. This particular seal was observed during one of the first experimental dives and the initial retardation of the heart was not as great as is usual after the seal is accustomed to the experimental procedure. In other respects the record of the heart is typical of many which have been observed. The interval between P and Q waves was usually prolonged from about 0.12 before the dive to 0.16 second in the later part of the dive. The condition of the Q, R, S waves was not appreciably altered. The T wave seemed to be gradually delayed during the dive until it followed R by about 0.2 second as compared with between 0.12 and 0.16 before the dive.

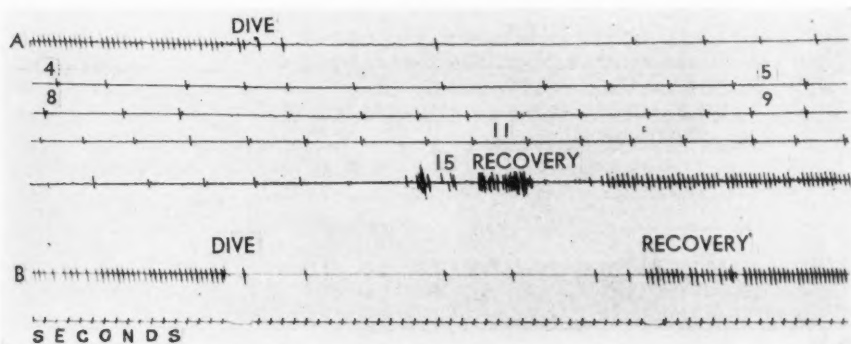


Fig. 1. Frequency of the seal's heart during dives, as recorded with the heart frequency counter. Second marks on bottom line fit both dives; signal refers only to the short dive. A. Sections from a 15-minute dive with numbers designating minutes after start. Marks at "15 recovery" result from struggling, not from heart beats. B. A 33-second dive.

This delay is somewhat obscured by the gradual change in form of the T wave which became conspicuous in the fourth minute of the dive and which produced an accentuation of the upward stroke of the T record. The change in the T wave persisted for some time during recovery. These changes in the electrocardiogram signify some alteration of the timing of events in the heartbeat, but they indicate that the duration of the systolic beat as shown by the electrocardiogram is not essentially altered during the bradycardia of diving. The observations of Scholander (1940) upon the seals *Halichoerus* and *Cystophora* are thus shown to be applicable to *Phoca*.

Pressure in the femoral artery. The record of pressure in the femoral artery taken with a Harvard rubber membrane manometer is indicated in figure 3, which reproduces sections of a record taken during an 8-minute dive. During the period before the dive only the signal of the beat is significant on account of the slow period of the recording system. Occasional spontaneous arrests in the

heart action of the seal at rest permitted the recorder to follow the arterial pressure changes in diastole. During some of these pauses before the dive the decline of pressure in a diastolic interval lasting 2 or 3 seconds was of the order of 30 mm. The pressure at the moment of diving was affected by mechanical disturbances extraneous to the circulation. During the period of the dive the usual bradycardia is shown and the mean level of blood pressure was practically unaltered from the level before and after the dive. During the long diastolic interval the pressure steadily declined, but the change of pressure was only of the

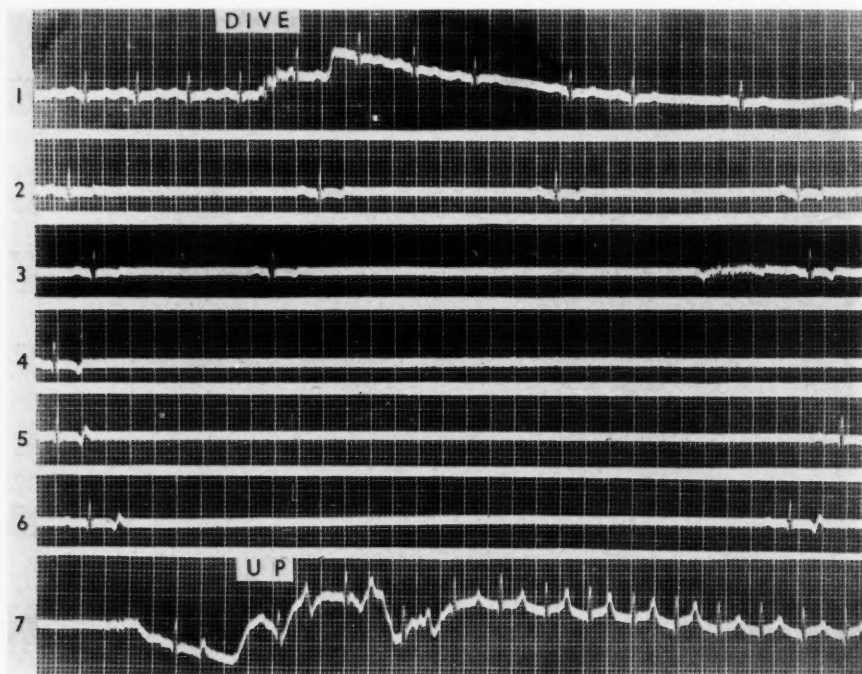


Fig. 2. Electrocardiograms of seal's heart at the start of a dive (1), at the beginning of the second minute (2), of the third (3), of the fourth (4), of the seventh (5), of the eighth (6), at the end of the ninth and the beginning of recovery after emergence (7). Heavy vertical lines mark fifth seconds.

order of 40 mm. and the lowest pressure observed at the end of a long diastolic pause was still about 100 mm. The slope of the diastolic pressure record was less steep during the long intervals than during the shorter intervals nearer the end of the dive although the difference in pressure was about the same. It appears that the rate of emptying of the large arteries during the prolonged diastole of diving is considerably retarded compared with the normal rate of emptying. In fact, the rate of emptying of the arteries appeared to diminish about in proportion as the frequency of the heart decreased, with the result that the mean pressure re-

maintained normal even during the long diastolic pauses. Records of this sort were secured in a number of observations of the pressure in the femoral artery of two seals weighing about 35 kgm. each.

In order to secure a direct measurement of the arterial pressure during a dive, the record shown in figure 4 was made with the mercury manometer. It is to be seen that at the moment when the dive began, the mean pressure in the femoral artery did not change appreciably and that this same mean level of pressure,

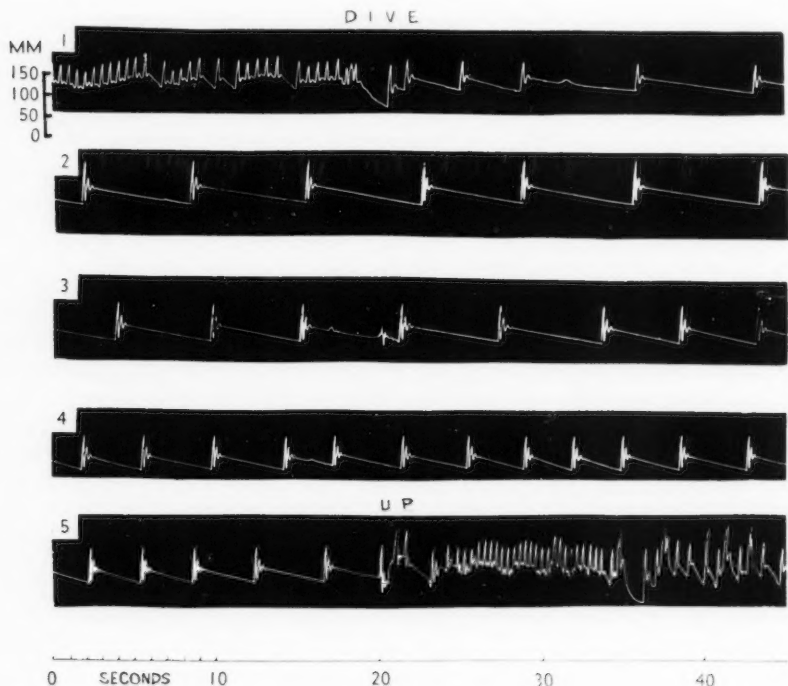


Fig. 3. Sections from continuous blood pressure record in the femoral artery during an 8-minute dive, recorded with Harvard rubber membrane manometer at the start of a dive (1), at the beginning of the second minute (2), of the fourth minute (3), of the sixth minute (4), at the end of the dive and in recovery (5).

around 130 mm., was maintained during a dive lasting for nearly 8 minutes. Following the dive a transient increase of pressure sometimes appeared but this increase was influenced by struggling and disturbance of the seal during emergence. The rapid systolic change is much distorted by the slow period of the recording system but the slow diastolic change during the dive reached the true diastolic level. The record thus confirms the indications of the membrane manometer and shows that arterial pressure remains around 110 mm. at the end of diastole during diving.

During the period of a dive when the heart is slowed to about 10 per cent of its normal frequency, the mean arterial blood pressure is, nevertheless, maintained at a relatively high level and near its normal value. It is unlikely that the amount of blood ejected by each stroke of the heart could increase ten-fold in order to maintain a steady output during the bradycardia and the record of the membrane manometer implies that the rate of emptying the large arteries slowed as the frequency of the heart diminished. It is natural to suggest that the slow frequency of the heart during diving finds compensation in a much constricted peripheral circulation. By the increased peripheral resistance emptying of the large arteries would be delayed during the long diastolic periods and normal pressure would be maintained in the main arteries.

Pressure in a peripheral vein. The pressure in a vein of the toe in the hind flipper of the seal was examined while practically at the same level as the femoral artery, but on the other side of the animal. A record of the pressure in a vein along one toe is given in figure 4. The level of venous pressure is the base line

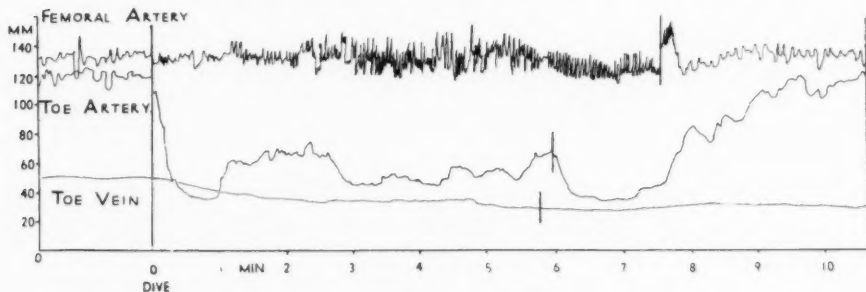


Fig. 4. Records of blood pressure with mercury manometer of a seal during dives taken from the femoral artery, a toe artery in the hind flipper about 1 mm. in bore and an adjacent small vein. The end of the dive in each pressure record is marked by a short vertical line.

above which the arterial pressure provides a force effective for the flow of blood through the tissues, and it may be seen that this venous base line remained at about the usual level during diving.

Pressure in a small artery. The pressure in an artery in the toe having a bore of about 1 mm. dropped immediately at the start of the dive to about the level of the venous pressure (fig. 4), and the pulse completely disappeared. Subsequently the pressure rose slightly but remained low for the remainder of the dive. Occasionally the slow pulse could be observed during the latter part of the dive, but it was usually obscured by other movements. After the dive the pressure remained low during the first part of recovery and did not begin to mount for one and a half minutes, and then only gradually approached the level of pressure in the femoral artery. The pressure in the toe artery during the dive was about at the level of venous pressure and practically abolished the arterio-venous pressure difference which could serve to maintain circulation in the flipper. It has been observed that arteries in the flipper often do not bleed when cut during

diving (Scholander, 1940), and it is likely that the constriction recorded in this artery would practically check all flow through its peripheral channels.

During recovery the pressure in the toe artery remained low for some minutes although the heart was beating rapidly. Inasmuch as the pressure in the femoral artery was at the usual level during recovery, the peripheral circulation was in general open, but the flipper continued to be closed off by the local arterial constriction. This is an example of a local delay in the opening of the circulation. It has been observed that the reoxygenation of muscles after a dive may be locally delayed (Scholander, Irving and Grinnell, 1941), and this delay would favor recovery in the tissues which are circulated and perhaps prevent the brain from injury by the sudden release into the circulation of a flood of lactic acid accumulating in the muscles during the dive (Scholander, 1940; Irving, Scholander and Grinnell, 1941b). This delay of local recovery might also be useful in deferring local recovery during the repeated short dives separated by a few breaths which seals often make (Scholander, 1940).

In another seal the pressure in a small artery of the hind flipper did not change appreciably during the dive but remained about at the normal level. We have observed visually, however, that constriction of the arterial circulation of the toe is of common occurrence during diving. Scholander (1940) has remarked upon the visible constriction of the small arteries during diving and upon the near cessation of blood flow through cannulae which were placed in those arteries. The same condition has been observed many times with *Phoca*, making it very difficult to draw blood from the small arteries during a dive whereas before and after the dive the flow through them was abundant. For these reasons we believe that the constriction of arteries in the toe to the point of complete closure is frequent and that it is perhaps typical of many small superficial arteries during diving.

Circulation in the mesenteric vessels. The mesenteric circulation was extensively observed in four animals and incidentally in several others. In a loop of exposed gut, it was seen that the size of the small arteries and veins gradually diminished during a dive until after 2 or 3 minutes the vessels were nearly bloodless in appearance. The infrequent pulse could be observed to move the larger vessels (normal diameter, 2 or 3 mm.) but not the smaller ones. The gut itself rapidly turned cyanotic in color during diving, and the reduction of vessel size evidently signified a great reduction or possibly even the arrest of blood flow through the mesenteries.

Quick constriction of arteries elicited by sensory stimulation. Almost any startling stimulus may reduce the blood pressure in the small artery in the toe of the seal. Examples are shown in figure 5 in which pinching, a sudden sound, or a visual stimulus quickly depressed the blood pressure, which then remained low for a considerable time after the brief stimulus. The result of stimulation by light is interesting because turning off the general illumination of the room caused a fall in blood pressure from which recovery occurred in about two minutes and while the light was still off. Turning on the light again produced the same fall in pressure. None of these stimuli bore any relation to respiration and the effects

appeared with the characteristics of reflex action. The reaction was quick and it was apparent within a few seconds of the time of the stimulus. The pressure fell practically to the venous level, as it did during diving, and it must likewise have resulted in the practical suppression of flow. The effect of the brief stimulus often persisted for several minutes, and repeated application of the same stimulus usually led to a diminished response. The quickness with which modifications of the constriction appeared shows that the control is not the automatic type of spinal reflex.

Sudden startling stimuli of the same sort may cause transitory retardation of the heart (Scholander, 1940), but the duration of reflex bradycardia of this sort is usually brief. It was occasionally observed, as, for example, during the prolonged depression caused by shouting, that the heart was beating rapidly while the peripheral blood pressure remained low. Evidently the constriction of the arteries which causes the drop in pressure is not always correlated with bradycardia. In other animals than seals, a sudden alarm may momentarily arrest

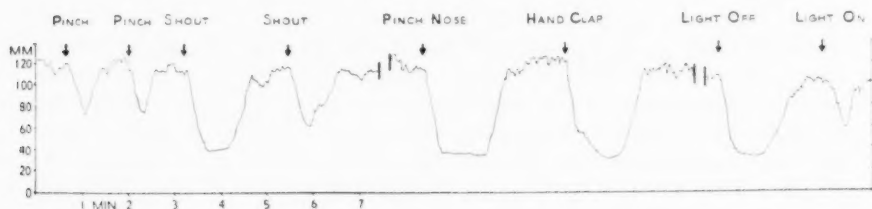


Fig. 5. Records of changes of pressure in the same toe artery as used for figure 4, in response to various stimuli of non-respiratory type. Each stimulus was momentary and applied at the time marked by arrow, except the light stimulus which was turned off and remained off steadily until turned on at the next arrow. Note that the other flipper was pinched to produce the first two recorded effects.

breathing and retard the heart action. These particular stimuli have no relation to respiratory conditions, but their action shows fine sensitivity of the vascular control.

The area supplied by arteries running to the flipper may well be among the most sensitive vascular regions, and the pronounced arterial constriction caused by alarm may not occur in all of the vessels of the seal. The hind flippers are practically non-muscular, and not covered with blubber, and their surface exposure and function so affect the conditions for heat loss that they may well require a specialized vascular control in the interests of temperature regulation.

The vasoconstriction of alarm which appears in the paling of the human face is also often limited in extent. That the vascular constriction of the seal caused by alarm is not widespread is indicated by the fact that the pressure in the femoral artery was not influenced by the stimuli which lowered pressure in the toe artery, as well as by the observation that the effect upon cardiac frequency is much more transient than is the reduction of pressure in the toe artery.

DISCUSSION AND CONCLUSIONS. Although at the start of diving the heart slowed 80 or 90 per cent, the pressure in the large arteries of the seal remained practically unchanged. At the time of emergence and when the heart accelerated and extensive alteration in the distribution of peripheral blood flow again occurred, the change in pressure was only small and transitory. It is evident that the control of the peripheral circulation of the seal must be very nicely coördinated in extent and time with the action of the heart in order to maintain an even level of arterial pressure.

With the demonstrated existence of normal arterial blood pressure during a dive, the circulation through a few tissues like the brain could be adequately maintained. Vascular changes of this nature have been shown before, for a reduction of circulation through the muscles and a sustained flow through the brain has been shown to occur when breathing is arrested in beaver and muskrats (Irving, 1937), cats, dogs and rabbits (Irving, 1938). It was then proposed that the control of the circulation would act during diving to conserve the oxygen supply for the use of such tissues as the brain (Irving, 1939). The observed situation in the diving seal shows that there is an extensive restriction of peripheral blood flow, and that in spite of the bradycardia the blood pressure is maintained at a normal level which could support a good cerebral circulation.

The practically complete suppression of the pressure difference between artery and vein in the flipper indicates that a large part of the restriction of blood flow through the flippers during diving often depends upon constriction of the arteries between the femoral artery and the artery in the toe. Very little further regulation of flow could be accomplished by constriction of the arterioles.

During recovery from the dive the pressure in the toe artery often remained low for several minutes although the heart was beating rapidly. It appears that the arterial constriction may occur independent of the change in frequency of the heart. The persistent arterial constriction during recovery was also local, because the pressure in the large arteries remained constant. In the usual reaction to diving, a large part of the peripheral circulation is probably constricted as a unit when the heart slows. The combined control of the heart and peripheral circulation can, as is shown in these experiments, be dissociated, and the arterial constriction in the flipper shows the independent operation of local vascular units, which in the massive reaction for diving are all controlled together.

Pinching, sound or light stimuli brought about a drop in pressure in the toe artery by arterial constriction. These reactions are not dependent upon the heart rate. They are subject to variation in time and in extent, in these respects resembling the reflexes which are controlled above the spinal level. The operation of arterial constriction shows a type of vascular control which can effect an elective distribution of the blood during diving. The arterial constriction was coördinated with the bradycardia so as to help maintain a steady pressure in the large arteries. What proportion of the elective restriction of blood flow is accomplished by arterial constriction is uncertain, but a function in the regulation of peripheral blood flow can be ascribed to the constriction of arteries. Constriction of the arteries would suitably regulate flow in large areas, but would not,

of course, permit the fine local differentiation of flow made possible by control of the arterioles.

SUMMARY

Although the heart of the seal slows during diving below 10 per cent of the resting frequency, electrocardiograms showed little change in individual heart beats. The pressure in the femoral artery remained at the normal level in spite of the bradycardia. An example of peripheral vasoconstriction is shown in the closure of an artery of the toe during the dive. This arterial constriction is apparently under reflex control and may be set in operation by many stimuli bearing no relation to respiration. Observed contraction of mesenteric vessels showed that there is a considerable reduction of their circulation during diving. These examples of peripheral vasoconstriction during diving along with others that are known indicate vascular adjustments which serve to maintain a normal arterial pressure which could maintain the circulation of a few tissues like the brain in spite of the extreme bradycardia.

Acknowledgment. Mr. Thomas H. Dorr of the United States Fish and Wildlife Service at Boothbay Harbor captured the seals and gave much assistance and excellent working facilities. Part of the expenses was provided by a grant from the Rockefeller Foundation for the study of the respiration of diving animals.

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SOME EFFECTS OF PROGESTERONE ON MALE AND FEMALE MICE¹

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Selye (1) has reported that progesterone injections cause an involution of the corpora lutea and marked ovarian atrophy in normal adult mice. Selye and Friedman (5) have shown that this hormone does not influence the seminiferous tubules in adult male mice and rats but does cause Leydig cell atrophy. Small doses do not affect the testes or accessory sexual organs of immature rats (7).

In the mature rat Selye, Browne and Collip (2) found that neither corpora lutea nor mature follicles were present but McKeown and Zuckerman (3) reported corpus luteum formation following progesterone treatment. Recently it has been shown that large doses of this material cause a marked follicular atresia but that ovulation and the formation of large corpora lutea with an increase in ovarian weight also occur (4). Robson (7) found that progesterone did not inhibit the ovarian response to human chorionic gonadotropin, but Astwood and Fevold (8) reported a decreased ovarian weight response to follicle stimulating hormone when the animals were pretreated with progesterone.

The experiments reported in this paper extend the observations on the effects of progesterone to include immature male and female mice. The effect of identical dosages on the mature and immature animals of both sexes was studied. The response of immature female mice to pregnant mare serum after treatment with progesterone was also investigated.

MATERIALS AND METHODS. Two strains of mice, the Hygenic and the Swiss strains were used. Each treated animal received 0.25 mgm. of progesterone² in 0.05 cc. of oil per subcutaneous injection for either a 10 or a 20 day period. If the injection period was of 10 days' duration, the hormone was administered every day, whereas in the 20 day periods of treatment, injections were made every other day. Thus, the total amount of hormone used was 2.5 mgm. in all experiments. All mice were sacrificed the day following cessation of treatment. Weights of gonads and accessory reproductive organs (intra-uterine fluid being

¹ The part of this investigation studied by one of us (JHL) was aided by a grant from the Rockefeller Foundation and administered by Dr. P. E. Smith.

² Crystalline progesterone "Proluton" and pregnant mare serum "Anteron" were supplied through the courtesy of Drs. E. Henderson and E. Schwenk of the Schering Corporation, to whom the writers are greatly indebted.

expressed) were taken. Histological sections of the tissues were prepared, the ovaries being sectioned serially.

RESULTS. Immature female mice. Mice 17 to 20 days of age were injected every other day with 0.25 mgm. of progesterone during a 20 day period. Increase in weight of the ovaries was suppressed by this treatment, although the difference in ovarian weight between the treated and control mice of the Swiss strain was not significant (table 1). Normal vesicular follicles of good size were observed in all the ovaries of injected mice but lutein tissue was absent. On the

TABLE 1
Effect of progesterone on female mice

NO. OF MICE	AGE AT START (DAYS)	TREATMENT	BODY WEIGHT AT AUTOPSY (GRAMS)	ORGAN WEIGHTS	
				Ovaries (mgm. \pm E _M)	Uterus (mgm. \pm E _M)
Immature female mice					
11S*	20	10 inj. of 0.25 mgm. in 20 days	21.2	4.0 \pm 0.3	28.7 \pm 3.1
17S	20	None	20.3	4.7 \pm 0.2	45.0 \pm 6.6
8H	17-19	10 inj. of 0.25 mgm. in 20 days	21.5	4.4 \pm 0.6	46.9 \pm 4.1
6H	17-19	None	20.7	7.1 \pm 0.2	89.1 \pm 12.3
3H	17-19	10 inj. of 0.25 mgm. in 10 days	12.5	3.1 \pm 0.4	20.3 \pm 1.2
3H	17-19	None	13.0	2.8 \pm 0.5	11.0 \pm 3.0
Mature female mice					
4S	127	10 inj. of 0.25 mgm. in 20 days	27.4	7.4 \pm 0.3	112.3 \pm 4.9
4S	127	None	28.1	13.1 \pm 0.7	96.2 \pm 4.6
10S	150-210	10 inj. of 0.25 mgm. in 10 days	28.9	11.3 \pm 1.2	99.2 \pm 9.6
12S	150-210	None	28.2	15.5 \pm 1.1	72.7 \pm 6.2
8H	30-37	10 inj. of 0.25 mgm. in 20 days	22.4	6.4 \pm 0.5	43.1 \pm 6.1
6H	30-37	None	23.7	8.2 \pm 1.2	92.0 \pm 22.0
11H	32-45	10 inj. of 0.25 mgm. in 10 days	19.6	4.5 \pm 0.7	54.0 \pm 9.2
8H	32-45	None	20.9	5.4 \pm 0.9	39.5 \pm 12.0

* Swiss mice = S; Hygenic mice = H.

E_M = mean deviation of the mean.

other hand, 50 per cent of the ovaries from untreated mice contained corpora lutea.

If the 2.5 mgm. total dose of progesterone was administered in a 10 day period, no significant effect was observed. Ovarian weight and histology were comparable in treated and control mice.

The trend of the uterine weights of those mice receiving progesterone in a 20 day period was generally lower than that of the control mice. However, following the shorter injection period the treated mice had a greater uterine weight than the controls.

Three litters of 22-day-old female mice were used to determine whether progesterone would alter the degree of ovarian response to pregnant mare serum (PMS). Eight mice received 0.25 mgm. of progesterone daily for 6 days. In addition, a total of 10 I. U. of PMS was administered subcutaneously at a different site in 3 equal injections at daily intervals starting the day of the 4th progesterone injection. Eight control mice received only the PMS and the injections were started on the 25th day of age. All mice were autopsied 24 hours after the last injection. Progesterone did not alter the ovarian weight response, the mean being 7.5 ± 0.6 mgm. in mice injected with progesterone and PMS as compared with 7.3 ± 0.1 mgm. following PMS treatment alone. The mean uterine weight was greater with added progesterone, being 53.6 ± 3.2 mgm. as compared with 39.9 ± 2.8 mgm. in the group treated only with PMS.

Mature female mice. Four of a single litter of 8 mature female mice were injected with 0.25 mgm. of hormone every other day for 20 days. Ovarian weight was significantly lower in the injected group (table 1). Large normal corpora

TABLE 2
Effect of progesterone on immature male mice

NO. OF MICE	AGE AT START (DAYS)	TREATMENT	BODY WEIGHT AT AUTOPSY (GRAMS)	ORGAN WEIGHTS	
				Testes (mgm. \pm E _M)	Seminal vesicle (mgm. \pm E _M)
11S	20	10 inj. of 0.25 mgm. in 20 days	20.8	144.6 ± 8.9	40.4 ± 7.3
14S	20	None	21.1	156.2 ± 3.2	70.0 ± 7.3
7H	20	10 inj. of 0.25 mgm. in 20 days	23.3	130.1 ± 4.7	$62.2 \pm 10.7^*$
4H	20	None	23.1	139.9 ± 5.3	$88.8 \pm 8.2^*$
5H	20	10 inj. of 0.25 mgm. in 10 days	13.7	57.2 ± 3.2	$12.9 \pm 1.1^*$
2H	20	None	13.7	61.3 ± 6.1	$8.1 \pm 3.0^*$

* Combined weights of the prostate and seminal vesicles.

E_M = mean deviation of the mean.

lutea and some follicles were present in the ovaries of the untreated mice. Normal vesicular follicles were observed in the ovaries of the progesterone treated mice but the corpora lutea were in varied states of involution. Very few large corpora were to be seen and, in general, they were reduced to approximately half normal size. Several corpora were markedly vacuolated. The ovaries of one mouse were virtually devoid of corpora lutea.

Administration of the hormone in a 10 day period also caused a regression in ovarian weight. However, the effect was less pronounced as compared with the 20 day injection period although the same total dose was administered. An apparent hastening of the involution of the corpora lutea had taken place in some cases, whereas other ovaries exhibited recent corpora lutea.

Immature male mice. Injections were started in these mice at 20 days of age and the hormone was administered in a manner identical to that used with the female. No significant influence of progesterone on testis weight was exhibited by either strain of mice (table 2). Spermatozoa were present in the

testes of all mice, treated and controls, after a 20 day injection period and sperm heads were observed after the 10 day injection period.

Seminal vesicle weights in control animals exceeded those of treated littermate animals (table 2). A similar effect was obtained on the accessory sexual organs of the Hygenic mice in which the combined prostate and seminal vesicle weights were compared. The glandular epithelium was not stimulated in either the prostate or seminal vesicle.

The injection of 2.5 mgm. of hormone in 10 days failed to prevent seminal vesicle weight decrease in castrate mature male mice. Injections were begun the day of the operation.

The weight of the adrenal and pituitary glands in male mice was not influenced by the progesterone injections.

DISCUSSION. Selye (1) noted involution of corpora lutea in mature mice treated with 1 mgm. of progesterone daily for 5 days. Daily administration of 10 mgm. over a 20 day period has produced ovulation and corpus luteum formation in the mature rat (4). In our experiments, definite involution of the corpora lutea and lowered ovarian weight was obtained in mature mice with a 2.5 mgm. total dose of progesterone injected over 20 days. This total dose injected in 10 days had less pronounced effect and corpora lutea formed in some cases.

When treatment was begun before maturity and extended to the age when corpora lutea normally form, not one of the mice had corpora lutea in the ovaries but follicular growth was present. Thus, progesterone injections suppressed corpus luteum formation but not follicular growth.

Progesterone failed to effect spermatogenesis in the immature male mouse even though injections were started 10 days before the initial presence of sperm in the testes. However, seminal vesicle weight increases were retarded in our injected mice. Selye (9) obtained lowered seminal vesicle weights in mature rats following treatment with large doses of progesterone.

Astwood and Fevold (8) concluded that progesterone suppressed the release of luteinizing hormone (LH) from the pituitary. The possibility that an influence on the secretion of LH is involved in the restoration of normal cycles in persistent estrus rats by progesterone injections has been suggested by Everett (10). The suppression of corpus luteum formation in immature female mice and the smaller seminal vesicle weights in immature male mice from the progesterone injections suggests that the treatment has diminished the secretion of luteinizing hormone.

SUMMARY

Administration of progesterone in an identical manner to immature male mice caused a retardation in seminal vesicle weight gain without influence on spermatogenesis. Similar treatment resulted in a suppression of corpus luteum formation without impairment of ovarian follicular growth in immature female mice.

In mature female mice subjected to the same treatment, ovarian atrophy and involution of the corpora lutea were observed.

Ovarian response to pregnant mare serum was not influenced by simultaneous injections of progesterone.

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THE RELATION OF HEAT PRODUCTION TO WATER METABOLISM DURING THE ADMINISTRATION OF SYNTHETIC THYROXINE¹

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It has been demonstrated by a number of workers (1, 3, 4, 5, 6, 7, 8)² that desiccated thyroid and anterior pituitary extract administration will intensify an already established experimental diabetes insipidus (d.i.) or unmask a latent d.i. In the main the available evidence indicates that this enhancement of the water exchange by these substances is due directly to their energy metabolism stimulating action.

The experiments reported here were undertaken 1, primarily to determine the daily correlation between the water exchange and the heat production both when metabolism is being stimulated by various substances particularly by thyroxine and during the decline following their administration, and 2, in an attempt to elucidate further the underlying mechanisms concerned in the augmentation of the fluid exchange in sensitive animals by certain metabolic stimulating substances.

EXPERIMENTAL PROCEDURES. The heat production was determined by the open circuit method essentially the same as that described by Bruhn and Benedict (2). Usually gas samples of 3 or 4 periods of 10 or 15 minutes' duration devoid of activity and preceded by at least 15 minutes of inactivity, were collected and analyzed. In case the results of the three or four periods did not agree by at least 5 per cent the average of the best agreeing was used as the day's metabolism.

The dogs rapidly became adjusted to the conditions of the experiment and rarely did activity complicate the collection of the gas samples. Food was withheld for at least 18 hours but never more than 22 hours before the run. Water was given ad libitum, the amount consumed being measured daily just before the run at 9 a.m. The amount of water drunk in the 20 to 22 hours preceding the metabolism determination is compared with the heat production obtained at the end of this period. The animals were maintained on a constant weighed diet and were housed in metabolism cages in a constant temperature room at 26°C. The rectal temperature of the animal was obtained just before the run.

¹ Aided by a grant from the University of Alabama Research Fund. A preliminary report of this work appeared in *This Journal* **126**: P448, 1939.

² Blotner and Cutler (*J. A. M. A.* **116**: 2739, 1941) have recently reported on the treatment of diabetes insipidus in the human by total thyroidectomy.

The desiccated thyroid (Lilly U.S.P.) and the dinitrophenol (Eastman) were given orally, the thyroid once a day, the dinitrophenol three times a day. The thyroxine (Roche Organon) was given intravenously, the anterior pituitary extract (Parke, Davis, Cornish)³ subcutaneously each once a day. Except in the case of the dinitrophenol the medication was given immediately after the metabolism run in the morning.

All operative procedures were performed by Dr. Allen D. Keller and detailed protocols of some of the individual experiments will be presented by him in a communication concerning the elaborating focus of the antidiuretic principle. The animals were rendered sensitive or otherwise by hypophysectomy in various degrees of completeness as illustrated in table 1.

TABLE 1

DOG NO.	LESION	24 HOUR WATER EXCHANGE*
1	Ordinary hypophysectomy**	50
2	Complete hypophysectomy plus deliberate encroachment on the hypothalamus†	350
3	Complete hypophysectomy‡	50
4	Ordinary hypophysectomy plus separation of stalk from hypothalamus	250
5	Complete hypophysectomy except for small strand of tuberalis	50
6	Complete hypophysectomy plus deliberate encroachment on hypothalamus	200

* Cubic centimeters of water per kilogram of operative weight.

** In an ordinary hypophysectomy the hypophyseal stalk is cut across through its distal extent such that all of the pars anterior and posterior lobe are removed but the proximal portions of the tuberalis and infundibulum remain attached to the hypothalamus.

† A very small remnant of tissue remained at base of pituitary fossa.

‡ Slight encroachment on the hypothalamus. A small remnant of tissue at base of pituitary fossa.

EXPERIMENTAL RESULTS. *Anterior pituitary extract.* The administration of anterior pituitary extract to dog 6 which previously had been given no medication, resulted in an elevation of the metabolism from 350 to 450 total calories and a coincident rise in the water exchange (fig. 1, 1b). The rise in metabolism and water exchange was not as marked as that obtained when either thyroid (fig. 1, 1a) or thyroxine was administered to the same dog. The shapes of the two curves, however, are remarkably similar, daily variations in metabolism being accompanied by deflections in the water exchange in the same direction. Cessation of treatment was followed by a slow return to the premedication levels.

Desiccated thyroid. Desiccated thyroid administered to dog 6 in a dosage of 2 grams daily for 10 days resulted in a 50 per cent rise in the total calories and a well marked increase of the water exchange from 250 to 550 cc. per kilogram of

³ The anterior pituitary extract was kindly furnished by Dr. Oliver Kamm, Parke, Davis & Co., and was from the same lot as that used by Keller (6).

body weight per day (fig. 1, 1a). Upon cessation of treatment both heat production and water intake returned to the premedication level within 8 days.

In sharp contrast thyroid administration to dog 5, an insensitive preparation, failed to increase the fluid consumption in spite of a greater percentile increase

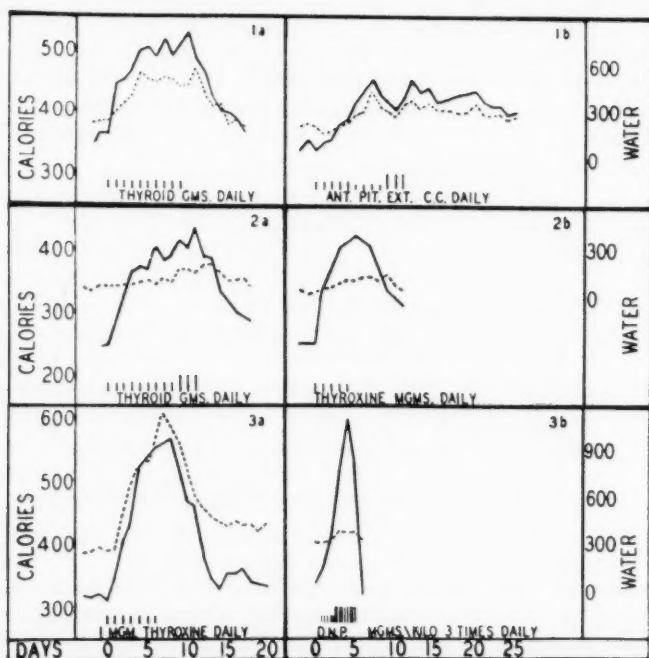


Fig. 1. Curves showing the 24 hour total heat production (solid lines) and fluid exchange in cubic centimeter per kilogram (broken lines) of hypophysectomized dogs during and after administration of metabolic stimulating substances.

1a, dog 6 given 2 grams of desiccated thyroid daily for 10 days (27 weeks postoperative).

1b, dog 6 given anterior pituitary extract 2 cc. daily for 5 days, 1 cc. daily for 4 days and 4 cc. daily for 3 days (34 weeks postoperative).

2a, dog 5 given 2 grams desiccated thyroid daily for 9 days followed by 4 grams daily for 3 more days (30 weeks postoperative).

2b, dog 5 given 1 mgm. thyroxine daily for 5 days (43 weeks postoperative).

3a, dog 4 given 1 mgm. thyroxine daily for 7 days (51 weeks postoperative).

3b, dog 4 given 1 mgm. dinitrophenol three times daily for five doses and 2 mgm. three times daily for 9 more doses (58 weeks postoperative).

in metabolism (fig. 1, 2a). It should be noted that animal 6 had a residual d.i. of 200 cc. while animal 5 manifested a normal water exchange.

Thyroxine. A series of normal dogs was given thyroxine in a dosage of 1 mgm. daily for five days without causing an appreciable rise in the water exchange. This same procedure was repeated on the hypophysectomized animal 5 (fig. 1, 2b) with essentially similar results in spite of a marked increase in the heat produc-

tion. Thyroxine administration to 1, 2, 3, 4 and 6, however, was accompanied by striking increases in the water exchange similar to that illustrated in figure 1, 3a, for dog 4. In all these cases (sensitive preparations) there is a similarity in the shape of the metabolism and water exchange curves.

Dinitrophenol. It seemed advisable to determine the effect of dinitrophenol on an animal which had given a good response to thyroxine. Consequently 1 mgm. of dinitrophenol three times daily for five doses and 2 mgm. three times daily for 9 more doses was given to animal 4 (fig. 1, 3b). Metabolism rose to the same extent as with thyroxine, but in spite of this stimulated metabolism the rise in water exchange is insignificant when compared to that obtained with thyroxine.

DISCUSSION. The foregoing experiments show that when the water exchange of sensitive animals is raised by anterior pituitary extract, desiccated thyroid, or thyroxine, there is a simultaneous increase in the metabolism. In all instances

TABLE 2

DOG NUMBER	CAL./KGM. BEFORE THYROIDINE	MAXIMUM CAL./KGM. AFTER THYROIDINE	CAL./KGM. INCREASE	WATER, CC./KGM. BEFORE THYROIDINE	MAXIMUM WATER, CC./KGM. AFTER THYROIDINE	WATER, CC./KGM. INCREASE	WATER, CC./KGM. INCREASE FOR EACH CAL./KGM. INCREASE
Animals having residual d.i.							
2	25.0	38.9	13.9	350	600	250	18.0
4	31.2	56.2	25.0	250	1100	850	34.0
6	38.7	56.2	17.5	200	800	600	34.3
Animals having no residual d.i.							
1	28.1	40.6	12.5	50	175	125	10.0
3	35.9	54.7	18.8	50	370	320	17.0
5	25.0	42.0	17.0	50	100	50	2.9

for any individual animal, the shape of the curves for heat production and fluid intake are similar in general outline (fig. 1, 1a, 1b, and 3a). This clearly demonstrates a close association of the energy metabolism with the water exchange that has been assumed to occur by previous investigators, although, with the exception of White, Heinbecker and Robinson, they actually did no metabolic studies.

In spite of this close association of the two curves for any given animal there is no quantitative correlation from one animal to another between the fluid and metabolic responses as is illustrated in table 2.

If one assumes that the three animals having a residual d.i. were totally anti-diuretic free, then it is obvious that the degree of rise in fluid exchange per unit rise in metabolism cannot be used in assaying the degree of anti-diuretic lack.

The experiments clearly indicate that the diuretic effect of desiccated thyroid is due to the metabolism raising principle, thyroxine. While it seems unnecessary to suppose that the diuresis is due to any other action of thyroxine than its stimulating effect on metabolism, yet, as was previously shown by White, Hein-

becker and Robinson an elevation of the metabolic rate *per se* does not necessarily lead to diuresis. This is demonstrated in the experiment illustrated in figure 1, 3a, 3b in which the heat production of an animal sensitive to thyroxine was raised with dinitrophenol without an appreciable rise in the water exchange.

SUMMARY AND CONCLUSIONS

The administration to "sensitive" dogs of anterior pituitary extract, desiccated thyroid and thyroxine caused an increase in energy production which occurred simultaneously with an increased water exchange. In view of the marked stimulating effect of synthetic thyroxine on the water exchange of animals with d.i., desiccated thyroid probably owes its diuretic effectiveness to no other action than its metabolism raising ability.

The lack of quantitative correlation between the rise in metabolism and the rise in fluid exchange between different animals of the group is discussed in view of the possible use of metabolic stimulants as test agents to assay the degree of antidiuretic deprivation.

Dinitrophenol, although a potent metabolic stimulant, was ineffective in elevating the water consumption of an animal which previously had been demonstrated sensitive to thyroxine, therefore an increase in metabolism does not necessarily lead to diuresis.

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THE METABOLISM OF CALCIUM AND PHOSPHORUS AS INFLUENCED BY VARIOUS ACTIVATED STEROLS

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The effects of massive doses of vitamin D on the metabolism of calcium and phosphorus have been the subject of numerous experimental (and clinical) studies. Because of the availability of highly concentrated preparations or crystals of vitamin D₂, attention has been largely confined to this substance. In general it has been shown that the administration of large doses of this vitamin to normal dogs causes an increase in serum calcium and phosphorus, an increase in the urinary excretion and a decrease in the fecal excretion of calcium and phosphorus, and a greater retention of these elements. Other substances known to be antirachitic and which would be expected to exert effects similar to those mentioned above include vitamin D₃, dihydrotachysterol (A.T.10), and Ertron.¹ Some of the effects mentioned are also characteristic of parathormone.

The literature on all of these substances has been carefully reviewed by Reed, Struck and Steck (1). A more comprehensive survey on A.T.10 through 1937 has been contributed by Holtz (2). More recently Harrison and Harrison (3) have compared the effects of parathormone and vitamin D on phosphorus metabolism and find that the latter causes an increase in the maximal rate of reabsorption of phosphorus in the tubules while parathormone has the opposite effect. Weber and Richardson (4) have shown that the administration of A.T.10 to human subjects causes an increase in urinary phosphorus excretion and an increase in the absorption of calcium, with higher serum calcium. Several articles seem to indicate that while both vitamin D₂ and A.T.10 cause an increase in the absorption of calcium and of the excretion of phosphorus in the urine, the effects of A.T.10 on the latter are more marked. This, for example, is the view of Albright, Sulkowitch and Bloomberg (5), who attribute the low antirachitic value of A.T.10 to this property. This hypothesis is in accord with the views of Shohl, Fan, and Farber (6) and of Shohl and Farber (7), who have shown (in rats) that non-toxic doses of A.T.10 will prevent the rickets induced by low-calcium high-phosphorus diets, while nearly toxic doses are required to prevent the rickets induced by high-calcium low-phosphorus diets.

Recently McChesney and Kocher (8) reported a preliminary study of the effects of various activated sterols on the serum calcium of albino rats. They found that corresponding antirachitic doses of crystalline vitamin D₂ and Ertron

¹ A form of irradiated ergosterol produced by the Whittier process and manufactured by the Nutrition Research Laboratories, Chicago, Ill.

are indistinguishable in their action; that vitamin D₃ causes a more prolonged hypercalcemia than does vitamin D₂; and that the effects of A.T.10 on serum calcium values are of the same order as that of 850 times its antirachitic equivalent of vitamin D₃.

Thus far no work has been reported which would permit a comparison of the effects of single massive doses of vitamins D₂, D₃, Erton, and A.T.10 on calcium and phosphorus metabolism as to: 1, duration and degree of changes in serum calcium and phosphorus; 2, changes in urinary output; 3, changes in fecal excretion; and 4, changes in total balances of these elements. It is our purpose in this paper to present such a study.

PROCEDURES. The experimental animals consisted of ten normal adult dogs. They were studied in groups of five and were kept in individual metabolism cages for the duration of each experimental period. Each day they received a specified amount, depending upon their weight, of a prepared dog food,² and any unconsumed residues were weighed the next day. The animals, with one exception (dog G, wt. 8.5 kgm.), always ate all of the ration offered except when, as a result of medication, anorexia developed. They were also given fresh spring water daily, *ad libitum*. The volume of the voluntary fluid intake was recorded.

For each test procedure mineral balances were determined during a seven day normal period, the animals having been put on the special ration several days before this control period was begun. The results obtained for the control period were compared with those for the first nine days following medication, which included the period of the greatest blood changes. The blood chemistry, however, was followed until the control values were definitely re-established. This set of animals was then allowed to rest while another experiment was being carried out on the other set.

Excreta were usually collected for periods of two days, although we occasionally used one or three day periods: the latter was quite satisfactory and reduced the time spent in routine analysis. Urine was collected under toluene. Feces were removed from the cages daily and placed in cartons until the total sample for the period had been collected. At the conclusion of the period of collection, aliquot samples of the urine and of the well-mixed feces were analyzed for calcium and phosphorus, thus giving the total output for the interval in question.

Methods of analysis. Urine, feces and food were wet-ashed by the method of Neumann (9). Calcium was determined by the standard Kramer and Tisdall procedure (10). Phosphorus was determined by the Fiske and Subbarow method (11). Serum calcium was determined by the Clark and Collip modification (12) of the Kramer and Tisdall procedure, and serum phosphorus by the Fiske and Subbarow method.

Medication. The various preparations, with one exception, were administered in sesame oil in no. 12 gelatin (veterinary) capsules. The contents of the

² Old Trusty Bovex, manufactured by Old Trusty Dog Food Company, Needham Heights, Massachusetts.

required number of capsules of Ertron, the active principle of which is dried on casein, were transferred to the larger capsules for administration.

The objective of the medication was to produce a maximal average rise in serum calcium of 4 to 5 mgm. per cent. Both Dale, Marble and Marks (13) and Goormaghtigh and Handovsky (14) have reported that the lethal dose of vitamin D₂ for dogs lies between 12 and 20 mgm. per kgm. McChesney and Kocher found that the dose required to produce (in rats) a hypercalcemia of the order we desired is about 12 mgm. per kgm. We therefore chose to give 5 mgm. per kgm. of both vitamins D₂ and D₃ since their hypercalcemic effects in rats are about equal, and this dosage of D₂ seemed to be sufficiently removed from the lethal level. The dose level of A.T.10 selected was also based on the observations of McChesney and Kocher; they found that the hypercalcemia resulting from the administration of 0.5 cc. of a 1 per cent solution of this preparation to a rat corresponded closely to that of 2.5 mgm. of vitamin D₃. An oral dosage of 0.1 cc. of a 10 per cent solution per kgm. was therefore administered to the dogs.

Studies of the fate of vitamin D in the rat (15) have indicated that a significant part of Ertron is not absorbed from, and that a part is destroyed in, the G.I. tract. An excess of 20 per cent, or a total of 240,000 units per kgm., was accordingly given in order that the systemic effect might be equal to that of the 5 mgm. dose of vitamin D₂.

RESULTS. *Composition of food.* A large number of samples of food were taken at various intervals prior to and during the experiments for the purpose of determining their calcium and phosphorus content. It was found that the calcium content varied from 0.30 to 0.40 per cent with an average value of 0.34 per cent. The phosphorus content varied from 0.28 to 0.35 per cent with an average value of 0.325 per cent. Since the dog food used was purchased in five case lots from a single shipment, we felt justified in basing our balance experiments on the assumption that over a period of seven or nine days the food for each group of dogs would average 0.34 per cent calcium and 0.325 per cent phosphorus. The validity of this assumption is strengthened by the fact that these exact values were obtained by averaging a number of composite samples taken on different days from the eight cans used. The calcium content of the drinking water was 36 mgm. per liter and the small amount from this source was included in the calculations of the dietary intake. The phosphorus content of the water, on the other hand, proved to be negligible and was disregarded. When Ertron was administered, the phosphorus content of the casein was added to the intake.

Analytical data. The data are presented in the form of four figures. Each figure shows the serum calcium and phosphorus (except in the case of Ertron where phosphorus was not done: see discussion) for the individual dogs, and the mean for the group. The urinary and fecal excretions, and the total balances of calcium and phosphorus are given as average values for the five dogs per day.

DISCUSSION. *Serum calcium.* All of the preparations tested elevated the serum calcium level within the first 24 hours post-medication. The action of A.T.10 was most prominent in that an increase of 3 mgm. per cent was noted

on each of the first two days. The other preparations caused an average rise of only 0.5 to 1.5 mgm. per cent per day. The observations of Holtz, who states (referring to human subjects): "In checking the serum calcium level it should be noted that the effect of a large dose of dihydrotachysterol is demon-

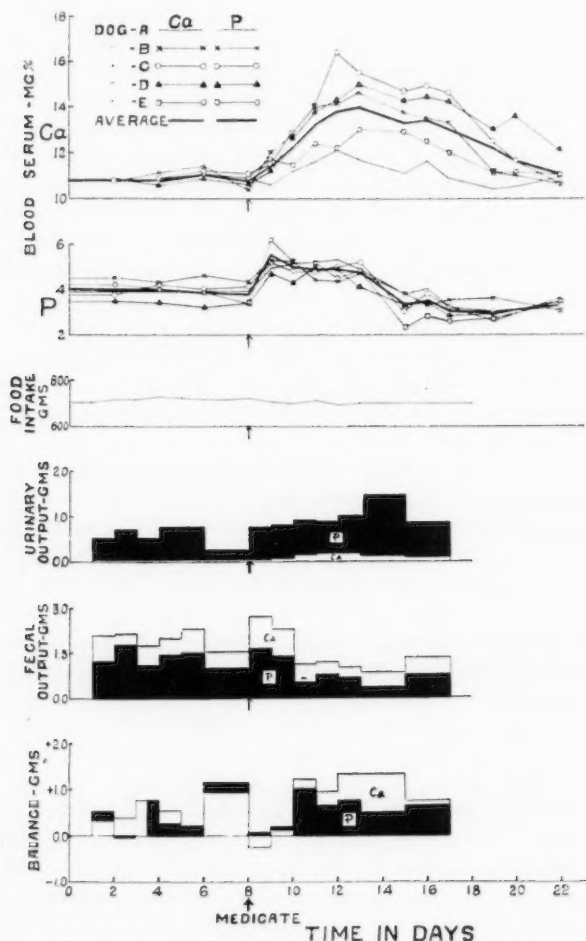


Fig. 1. Results of medicating five adult dogs with 5 mgm. of crystalline vitamin D_2 per kilogram of body weight.

strable at the earliest after 2 or 3 days, and that the maximal effect is reached between the 4th and 7th day," are not in agreement with the very short latent period noted after medication of normal dogs as reported in this paper. In point of time the peak calcium levels after A.T.10, vitamin D_3 , vitamin D_2 ,

and Ertron were noted at 3, 4, 5 and 5 days post-medication respectively. This is the same order in which the peak responses were observed in rats by McChesney and Kocher, but in each case they are delayed by about 48 hours.

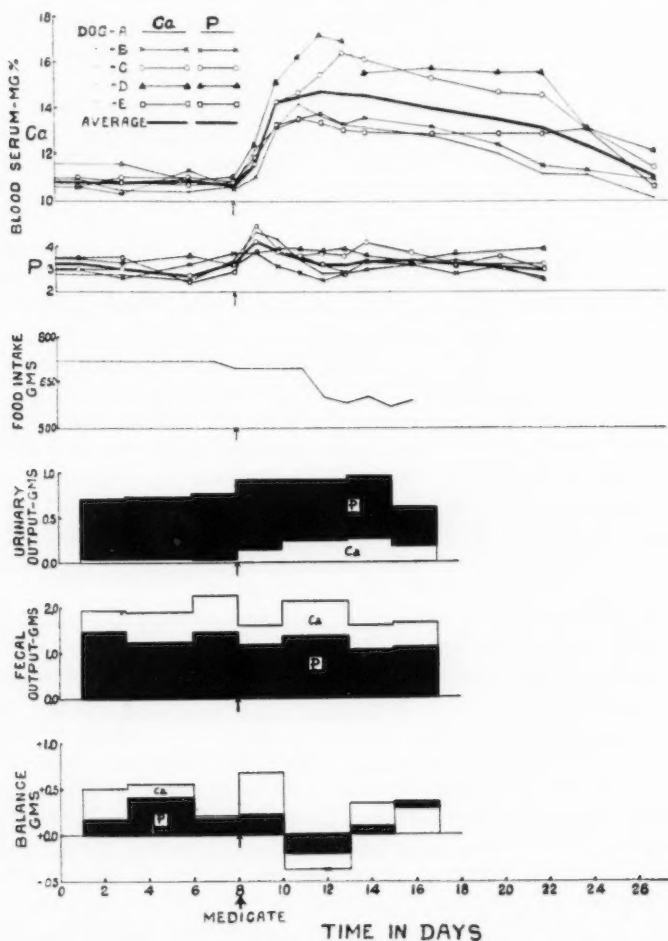


Fig. 2. Results of medicating five adult dogs with 5 mgm. of crystalline vitamin D_2 per kilogram of body weight.

The responses to vitamin D_2 and Ertron are considered to be practically identical. Both gave their peak values at the same interval after medication and, although the serum calcium averaged higher after Ertron, the difference is probably without significance in view of the fact that a different set of dogs was used for this test.

Still a third type of response was obtained with vitamin D₃. The initial rise was more rapid than with vitamin D₂, but slower than with A.T.10. The peak value in the different animals was reached from the second to the fifth day (average 3.5 days), followed by an exceedingly slow decline. Two dogs, for example, showed no appreciable change from the sixth to the fourteenth days after medication. The average value for the entire group decreased only 1 mgm. per cent from the fourth to the sixteenth days post-medication.

The effects of all these preparations are markedly different from those of parathormone on normal dogs. A dose of parathormone sufficient to cause an increase in serum calcium of 5 mgm. per cent has its maximum effect at about the sixteenth hour and by the thirty-sixth hour essentially normal conditions are restored (16).

The responses of the individual animals to these preparations were quite irregular. The greatest uniformity of response was obtained during the first two days of the A.T.10 medication, but wide variations were noted after the second day. The net serum calcium increases or peak values also varied maximally. Thus the extremes represented by two animals after Ertron were 13.7 and 19.8 mgm. per cent. The greatest irregularity of individual response was also noted after Ertron where one animal showed three distinct maxima before the base level was again reached. The only generalization which can be made with regard to individual response is that an animal which gives a maximal response to one medication also gives a maximal response to the others, and vice versa. Dale, Marble, and Marks have suggested that the response depends more upon age than upon weight. On the other hand, the degree of loss or destruction in the G.I. tract may be the most important variable.

Serum phosphorus. These values were determined only for vitamins D₂, D₃ and A.T.10; since both Ertron and vitamin D₂ are forms of irradiated ergosterol it was deemed unlikely that the reaction to Ertron would differ significantly from that to D₂. As to the results, the only conclusions that may be drawn from these observations are as follows: significant changes occur in the first 24 hours post-medication, when a considerable elevation is noted. Normal values are essentially restored by the end of 72 hours except after vitamin D₂, where slightly elevated values persisted up to five days. Later, during the period in which the serum calcium was returning to normal, the phosphorus tended to be subnormal. This tendency was particularly prominent after the A.T.10 medication.

Anorexia. Some degree of anorexia, associated with hypercalcemia, was observed after all of the medications. In general the animals lost their appetite when the serum calcium reached a level of 17 to 18 mgm. per cent depending on the individual. After vitamins D₂ and D₃ only one animal (the same one) was affected; after Ertron and A.T.10 all of the animals but one were affected. The most severe anorexia was found after A.T.10 but it lasted for only four days coincident with the highest serum calcium levels. After Ertron the anorexia was less severe than after A.T.10 but it persisted for a week, and the animals could be induced to eat then only by changing the ration to fresh ground beef.

During this week one animal ate only 160 grams of food, about 2 per cent of its usual intake.

Urinary output. The urinary calcium output was materially increased after medication with each of the preparations tested. An increased output of phos-

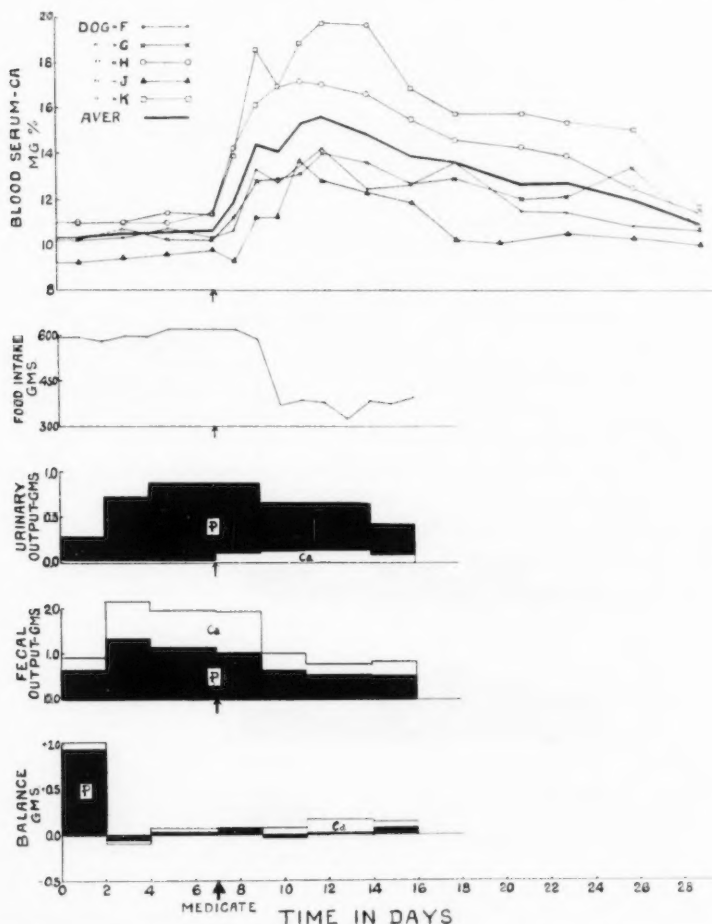


Fig. 3. Results of medicating five adult dogs with 240,000 units of Ertron per kilogram of body weight.

phorus followed medication with vitamins D_2 and D_3 . After Ertron and A.T.10 no apparent change in phosphorus occurred. However, it must be recalled that following these medications the dietary intake of phosphorus was greatly restricted due to the refusal of food; therefore it can be stated that no decrease in output took place when one would normally have occurred. The increase in

calcium output after these two preparations took place in spite of the restricted intake.

Fecal output. The normal fecal calcium and phosphorus values were materially decreased by all of the preparations tested. The decrease noted was most

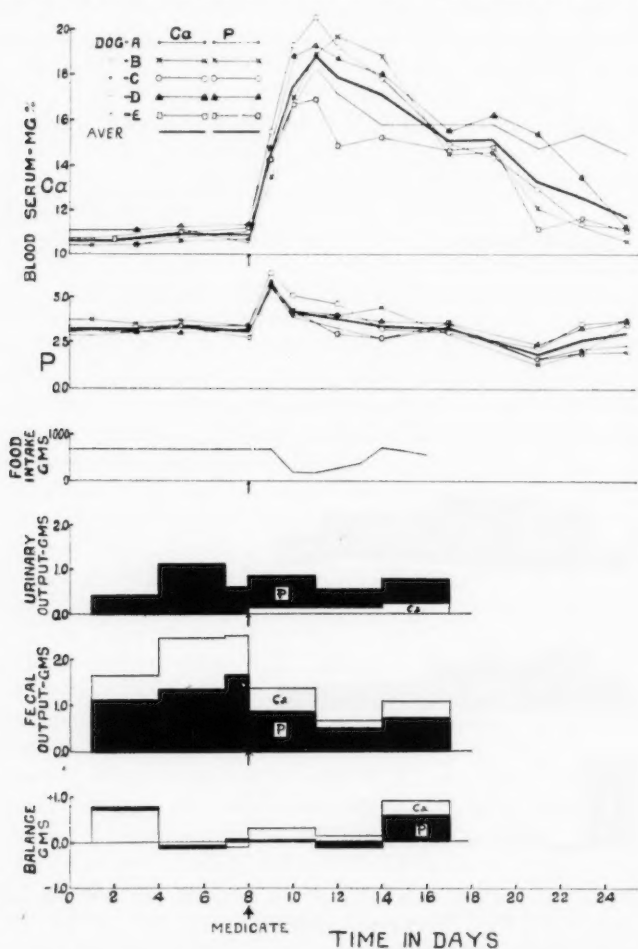


Fig. 4. Results of medicating five adult dogs with 0.1 cc. of a 10 per cent solution of A.T.10 (equivalent to 2 cc. of commercial A.T.10) per kilogram of body weight.

significant after vitamins D_2 and D_3 since in these cases there was no accompanying restriction of dietary intake. It should also be noted that under the conditions of the test vitamin D_3 had a laxative effect which might be expected to diminish absorption and lead to a temporarily increased output. In spite of this, a decreased output was actually observed.

Total balances. Variations in fecal output from day to day tend to give similar fluctuations in calcium and phosphorus balances. Although the dogs ordinarily passed some feces in every 24 hour period, they did not always do so. Variable food intakes accompanied by practically undiminished output also produce fluctuations in balances. However, the fact that we averaged data on

TABLE 1

Comparison of effects of various activated sterols on the calcium and phosphorus metabolism of dogs

FACTORS STUDIED	CHARACTERISTICS OF RESPONSE	VITAMIN D ₂	VITAMIN D ₃	ERTRON	A.T.10
Serum calcium	Time of peak value	5th day	4th day	5th day	3rd day
	Average peak value	13.9 mgm. %	14.7 mgm. %	15.6 mgm. %	18.6 mgm. %
	Individual peak value	16.3 mgm. %	17.3 mgm. %	19.8 mgm. %	20.8 mgm. %
	Average duration of hypercalcemia	11 days	17 days	20 days	15 days
	Rate of rise	Slowest			Fastest
	Rate of fall		Slowest		Fastest
Serum phosphorus	Time of peak value	1st day	1st day		1st day
	Average peak value	5.2 mgm. %	4.2 mgm. %		5.9 mgm. %
Food consumption	Incidence of anorexia	20% of dogs	20% of dogs	80% of dogs	80% of dogs
	Duration of anorexia	3 days	6 days	7 days	4 days
	Intensity of anorexia	Least			Greatest
Urinary excretion of	Calcium	Increased	Increased	Increased	Increased
	Phosphorus	Increased	Increased	Unchanged	Unchanged
Fecal excretion of	Calcium	Decreased	Decreased	Decreased	Decreased
	Phosphorus	Decreased	Decreased	Decreased	Decreased
Total balance of	Calcium	Increased	Decreased	Increased	Increased
	Phosphorus	Unchanged	Decreased	Increased	Decreased

TABLE 2

Effect of various activated sterols on calcium and phosphorus balances
Daily balances*—grams

ELEMENT	PERIOD	VITAMIN D ₂	VITAMIN D ₃	ERTRON	A.T.10
Calcium	7 days, pre-medication	+0.58	+0.43	+0.02†	+0.27
	9 days, post-medication	+0.86	+0.15	+0.22	+0.44
Phosphorus	7 days, pre-medication	+0.56	+0.27	-0.01†	+0.28
	9 days, post-medication	+0.54	+0.09	+0.06	+0.19

* Average of 5 dogs per day.

† 5 days pre-medication.

five animals for periods of two or three days reduced these fluctuations to a considerable extent.

We were able to confirm that vitamin D₂ causes an increased retention of calcium; i.e., a more positive balance. There was no appreciable change in phosphorus balance. Vitamin D₃, on the other hand, appeared to cause a lesser retention of these elements. While the balances remained positive, they were

not as favorable as during the control period. In the case of Ertron the first two days of the control period should probably be disregarded on the ground that equilibrium had not yet been achieved. (This was the first experimental procedure to which the dogs had been subjected.) With that reservation, the medication did result in some improvement in mineral balance in spite of the restricted intake. A.T.10 was found to give a more favorable calcium, but less favorable phosphorus balance. This is in accord with what would be expected from the statements in the literature to the effect that this preparation causes a loss of phosphorus from the body although here the loss occurs only in the sense that less of the amount available is retained.

The more important numerical data are presented in Tables 1 and 2. The latter table compares mineral balances in the pre-medication period with those from the post-medication period since the average daily balances are not in every case readily determined from an examination of the figures.

SUMMARY

The effects of single massive doses of vitamins D₂, D₃ and of Ertron and A.T.10 on the calcium and phosphorus metabolism of dogs have been compared. As to serum calcium, vitamin D₂ and Ertron are essentially the same in their effects. Vitamin D₃ is characterized by the long persistence of a rather moderate degree of hypercalcemia which follows its administration. A.T.10 causes a very rapid rise of serum calcium followed by a comparatively rapid fall. All of the products cause a rise in serum phosphorus (Ertron not studied). All of the products decrease fecal and increase urinary output of calcium. They also decrease fecal output of phosphorus and either increase urinary output or maintain it at a constant level in spite of decreased intake. All of the products except vitamin D₃ improved calcium balances; Ertron improved the phosphorus balance slightly.

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COPPER-INDUCED PSEUDOPREGNANCY IN THE ADULT ESTROUS RAT

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It has been adequately demonstrated that sterile coitus, mechanical or electrical stimulation of the cervix uteri, and electrical stimulation of the brain stem will induce pseudopregnancy in the cat, ferret, rabbit and rat (1). Emmens (2) and Friedman (3) employed intravenous injections of copper salts to induce ovulation and pseudopregnancy in the estrous rabbit. Brooks (4) reports that pituitary stalk section prevents the ovulation in the doe that would ordinarily follow coitus or intravenous copper injections. His experiments indicate that in the estrous rabbit both stimuli are mediated over a similar neuroendocrine path.

Several investigators, however, have reported that estrous cycles continue in rats in which the pituitary stalk has been sectioned (1, 5, 6); but it is uncertain that these lesions prevented the induction of pseudopregnancy by coital stimuli. Since pregnancy and pseudopregnancy in the rat, cat, ferret and rabbit are quite likely dependent upon a fundamentally similar neuroendocrine physiology (7), intravenous injections of copper salts might be expected to induce pseudopregnancy in the estrous rat. This paper cites experiments which test this possibility.

MATERIALS AND METHODS. A 1 per cent solution of copper acetate [$\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot \text{H}_2\text{O}$] has a copper ion concentration of approximately 3.0 mgm. per cubic centimeter of solution. The pH of this solution is approximately 5.4. By adding a 2 per cent solution of copper acetate to an equal volume of a 2 per cent solution of sodium acetate, the final solution is brought up to pH 5.9–6.1. This was the source of our copper ion for all the experiments reported herein.

The rat was lightly anesthetized with ether and then placed on its back. The medial aspect of the thigh was shaved, and an incision made through the skin and mammary fat along the outline of the femoral vein into the inguinal region. This exposed the femoral vein at its junction with the inferior mammary vein. At this point the vein is large enough so that it can be punctured easily with a 30-gauge needle. The needle can be observed in the vein and there is no loss of injection medium into the surrounding tissues. The procedure can be carried out in 5 minutes under ordinary asepsis. We have used the same rats several times for similar injections.

The rats used in these experiments were of a Wistar strain, bred and raised in our stock colony. They were removed to the experimental colony when 80 to

200 days old and maintained on a stock diet, which included a dry basal ration supplemented twice weekly with lettuce and carrots. Six to 8 rats were kept in a large cage. Individual daily vaginal smear records were made throughout the investigation until the animals were sacrificed. Rats were used only after they had exhibited two or more normal estrous cycles. All injections were given intravenously shortly after the reading of an estrous vaginal smear, unless otherwise stated.

RESULTS. In the course of the experiment 16 rats in estrus were injected with 0.1 cc., and 10 estrous rats were injected with 0.15 cc. of the copper solution. A prolonged diestrous vaginal smear (which ranged from 9 to 19 days) was induced in all the above rats following the intravenous copper injection. To be sure that

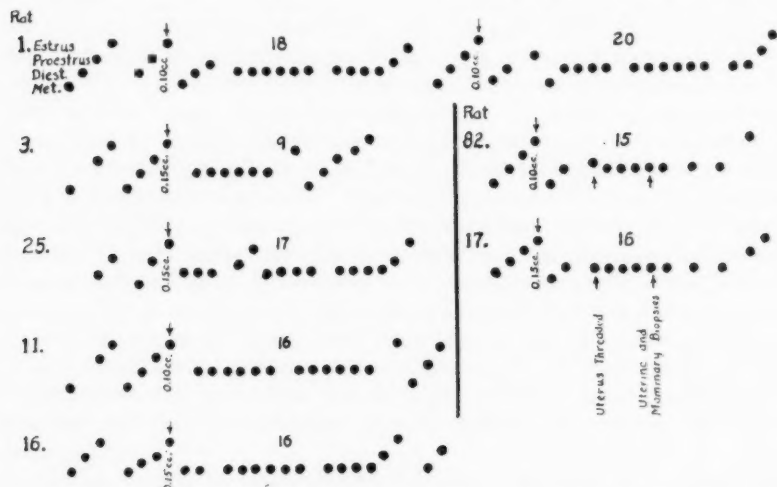


Fig. 1. Prolonged diestrus (pseudopregnancy) induced with 0.1 cc., or 0.15 cc. of a 1 per cent copper acetate solution given intravenously in normal, regularly cycling adult female rats. Each dot represents a daily vaginal smear observation. ↓ indicates injection at estrus. The number indicates the days duration of prolonged diestrus.

this prolonged diestrus represented a true pseudopregnancy, the uterine horns of several rats were traumatized with silk sutures on the fourth day of the induced diestrus. Definite deciduomata were found in the uteri on the eighth day; and biopsies of the mammary gland showed an early stage of proliferation. Figure 1 shows the protocols of daily vaginal smears preceding, and subsequent to intravenous injections at estrus of several of the above rats.

Six rats were injected with 0.1 cc. copper solution at the time of a late estrous, or an early metestrous vaginal smear. The smear records on these rats show a prolonged diestrus interrupted once on the fourth to sixth day by an apparent proestrous or estrous smear (rat 25). Intravenous injections of 0.1 cc. of copper solution given to 13 rats in metestrus and 14 rats in diestrus stages of the cycle failed to induce a prolonged diestrus. Only 5 of these rats experienced a 2- or

3-day alteration in the rhythm of their first cycle immediately following the injection. Apparently copper is effective only during the preovulation phase of estrus.

The operative procedure was duplicated using distilled water instead of the copper solution in three control experiments. There was no alteration of the estrous rhythm in these trials. Also, 0.15 cc. of the copper solution injected subcutaneously in 5 estrous rats was ineffective in inducing pseudopregnancy. However, ulceration and necrosis were produced at the injection sites. Figure 3 presents the vaginal smear protocols of a few of these rats. Pfeiffer (8) reports similar trauma following subcutaneous injections of a 10 per cent copper solution which did not produce any changes in the ovarian histology of immature rats.

Since this intravenous method in the estrous rat is a new procedure, it appeared that a valid comparison with the intravenous copper injection would be the

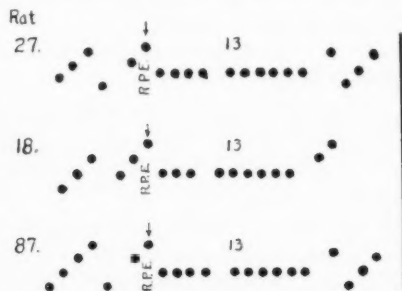


Fig. 2

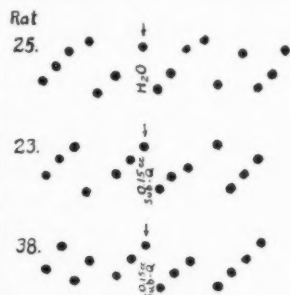


Fig. 3

Fig. 2. Prolonged diestrus (pseudopregnancy) induced with rat pituitary extract (R.P.E.) in normal, regularly cycling adult rats. Each dot represents a daily vaginal smear observation. ↓ indicates injection at estrus. The number indicates the days duration of pseudopregnancy.

Fig. 3. The protocols of an adult cycling rat injected intravenously with 0.15 cc. distilled water, and two other adult cycling rats injected subcutaneously with 0.15 cc. of the copper solution. Each dot represents a daily vaginal smear observation. ↓ indicates injection at estrus.

demonstration that pseudopregnancy could also be induced with a gonadotropic extract. Rat pituitaries were desiccated in acetone, and then air dried. The powder was extracted with distilled water made slightly alkaline to phenolphthalein with 1/10 N NaOH; and sodium chloride was added to make the solution physiologically normal. A single intravenous injection of a dose representing 1.0 mgm. of fresh pituitary from castrated rat induced typical pseudopregnancy (fig. 2).

Since copper solutions are known to be toxic, it was of interest to determine the minimal effective dose that could induce pseudopregnancy, and also to determine the lethal dose.

Two estrous rats were injected with a dose of 0.025 cc. of the copper solution, 2 others with 0.05 cc., 2 more with 0.06 cc., and 1 with 0.08 cc. The estrous rhythm of these rats was not altered. But intravenous injections of 0.1 cc. and

0.15 cc. of the copper solution induced a pseudopregnancy in all the trials. Therefore 0.10 cc. is the minimal effective dose of our copper solution.

Lethal results were obtained in 2 rats within a few minutes following the intravenous injection of 0.6 cc. of the copper solution. A severe diarrhea was induced in 3 rats within a few minutes after the injection of 0.4 cc. of the copper solution. These rats died within a period of $\frac{1}{2}$ to 2 hours after the injection. The minimal lethal dose appears to be approximately 0.3 cc. of copper solution. The rats injected with this dose were semicomatose when the ether effects should have worn off; there was a hematuria; and they were moribund 48 hours after the injection. The minimal effective dose of 0.1 cc. copper solution injected intravenously occasionally induces a transient hematuria for a few hours, but no other injurious effects were noted.

In the course of similar experiments with estrous rabbits it has been found that doses of 1.0 cc. of this same copper solution induced ovulation whereas 0.75 cc. was ineffective. Doses of 5 cc. to 7 cc. were lethal for rabbits.

SUMMARY

Intravenous administration of copper solutions induce pseudopregnancy in the adult estrous rat. The minimal effective dose is 0.1 cc. (0.3 mgm. of copper ion) of a 1 per cent copper acetate solution. Approximately 1 cc. of the same solution is the minimal ovulating dose in the estrous rabbit.

Pseudopregnancy is also induced in the adult estrous rat by intravenous injection of rat pituitary extract. These data suggest that the copper may act through the pituitary since the reports in the literature indicate that the intact pituitary is necessary to mediate the copper induced pseudopregnancy in the rabbit. They also suggest that a fundamentally similar neuroendocrine physiology exists in both the spontaneously ovulating rat and the non-spontaneously ovulating rabbit.

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RELATIVE INCREASE IN CHLORIDE EXCRETION IN THE DOG AFTER GRADUATED DOSES OF MERCURIAL DIURETICS¹

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Since mercurial diuretics are extensively used clinically to increase salt and water excretion, the purpose of this study was to find a more quantitative method of evaluating the chloride excretion produced by the organic mercurials and to determine any quantitative differences between these diuretics. Because mercurial diuretics increase urine flow by an inhibition of tubular function (1), it was thought probable that a range of dosage between minimum and maximum tubular inhibition could be determined for each mercurial. This range in dosage, as determined in dogs with bladder fistulae by increasing the dose of mercurial each week, would then represent the safe or "physiological range" of dosage and would be bounded on one extreme by the first evidence of increased chloride excretion and on the other extreme by the maximum chloride excretion obtained. The above approach would provide data on the physiological limits of chloride excretion which can be obtained with progressive tubular inhibition.

EXPERIMENTAL. Preliminary studies indicated that the total chloride excretion in milligrams of NaCl² would afford the most exact criterion of the total diuretic action. This was based on the finding that in order to obtain constant results, dehydrated animals must be used. Such animals, when injected with mercurial diuretics, frequently respond with a marked increase in chloride excretion, but no increase in water excretion. If urinary volume alone is used as a criterion of diuresis, many effective therapeutic responses would remain undetected.

The mercurial diuretics used in this study were: Salyrgan (N.N.R.), Mercurin (N.N.R.), the mercuric base of Esidrone (Na salt of pyridine-dicarboxymercuri-hydroxy-propylamide), and their corresponding Theophyllin-containing compounds, Salyrgan-Theophyllin solution (N.N.R.), Mercupurin (N.N.R.), and Esidrone.

Six groups of 5 dogs with bladder fistulae (2) were used in these studies. Experiments were performed by increasing weekly the mercurial dose in each group of 5 dogs. The animals were used only once a week to allow recovery of salt balance and to minimize the cumulative poisoning. The dogs were all well trained and unanesthetized. Water was withheld from them for 5 hours before they were placed in stocks, and a control period of 30 minutes was allowed, during

¹ Aided in part by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

² The product of the urinary volume and the milligram NaCl per cubic centimeter.

which time urine volume and chloride determinations (expressed as NaCl) were made every 10 minutes. At the end of the control period the diuretic drug was administered slowly, intravenously in a 1 to 3 cc. volume. Urine volume and chloride determinations were made thereafter every 10 minutes for 2 to 2½ hours. Inasmuch as this was also a study in chronic toxicity, the same approximate number of weekly doses was given to each group of animals. The increment of dosage was estimated from the therapeutic index³ of each compound, so that each group of dogs received approximately 6 injections. In general, the experiments on each group of dogs were terminated when a larger dose of the mercurial diuretic produced a definite decrease in chloride excretion. Clinical methods of studying renal toxicity were not applicable in these dogs, for the exposed bladder wall frequently exudes mucin and red blood cells which invalidate the usual clinical tests. The results of these studies are tabulated in figure 1.

DISCUSSION. Cardiac death (3) is known to occur with toxic doses of Salyrgan in the dog. The only cardiac deaths in this study occurred with Esidrone, where 2 dogs died after doses of 4 mgm. Hg/kgm. The addition of Theophyllin either in chemical combination (3 per cent in Mercupurin) or as a partial mixture (5 per cent in Salyrgan-Theophyllin solution) increases slightly the tolerated dose producing maximum inhibition and aids in reaching a higher peak of chloride excretion. All of the six groups of dogs showed a final decrease in chloride excretion with the highest dose used. This probably indicates that the physiological mechanism which accounts for this type of diuresis had been exceeded,—that is, the tubular inhibition had been superseded by tubular or glomerular damage.

Tubular damage (4) following repeated therapeutic doses of Novasurol has been observed. This was more marked following larger doses and disappeared if the drug was discontinued. In the 32 dogs given large doses of mercurials in these studies all of the kidneys were fixed one week after the last dose of mercury, and when stained with scarlet red, showed histological evidence of fatty degeneration of the tubules. There was also minimum fatty infiltration of the glomeruli at these toxic levels of dosage. No quantitative difference in kidney damage was noted; nor were any differences anticipated, since each group of dogs had been given a planned series of doses the last of which probably exceeded the point of maximum tubular inhibition as evidenced by the decreased chloride excretion obtained.

³ Determined by intravenous rat toxicity and dog diuretic studies.

Fig. 1. The milligram chloride excretion, expressed as NaCl, has been corrected, to a theoretical 10 kgm. dog (ordinates). Time is given in minutes (abscissae). Note that the doses of each mercurial have been increased stepwise until a larger dose produces a decreased chloride excretion. The abnormal range in control chloride excretions in the Salyrgan-Theophyllin solution graph may have been due to a high environmental temperature, as these experiments were performed in the late summer and early fall. Note the initial inhibition of chloride excretion with the mercuric base of Esidrone. Also note the speed of onset, the high level of chloride excretion, and the similarity of the curves obtained with Mercurin and Mercupurin. The addition of Theophyllin to Salyrgan and Esidrone results in an increase in chloride excretion.

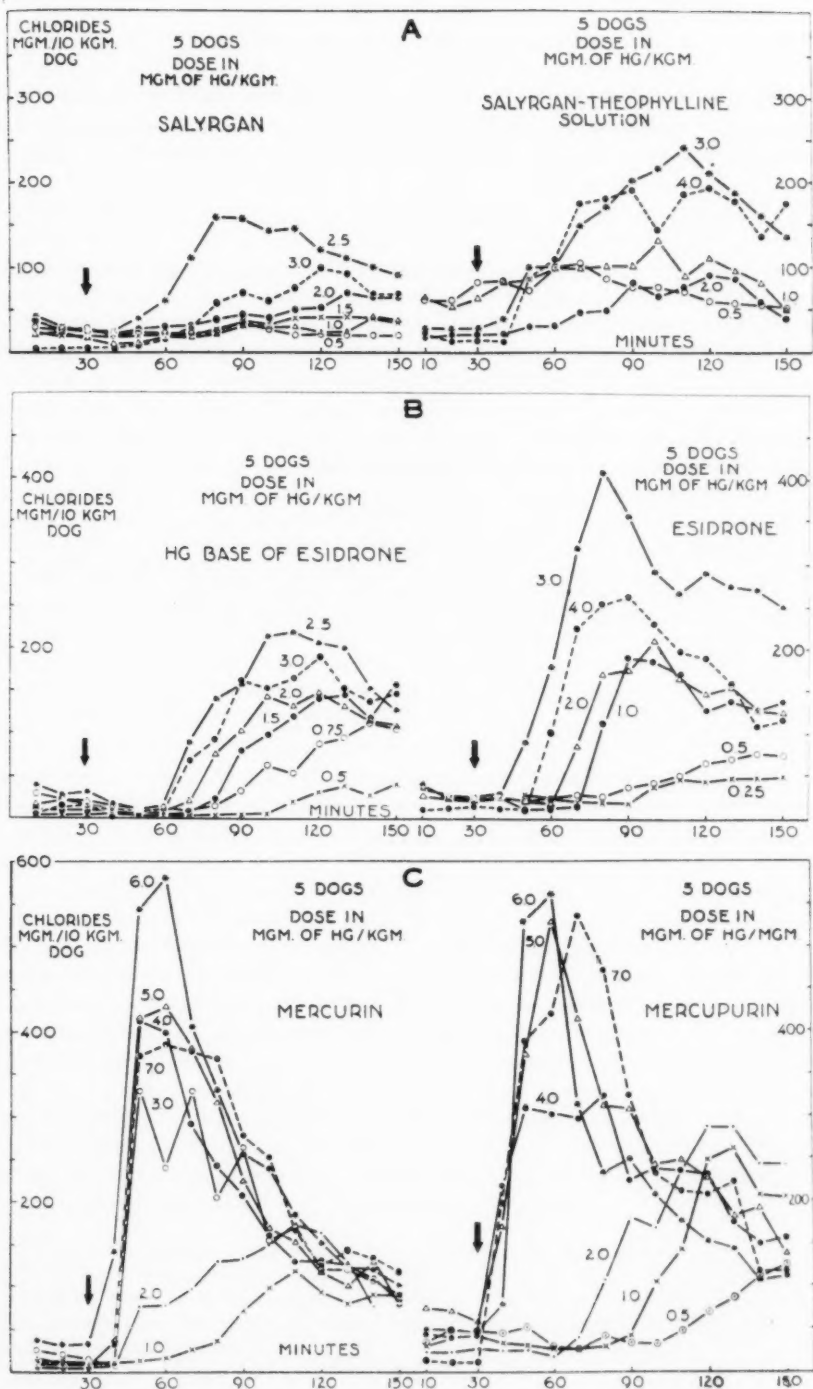


Fig. 1

The deaths which occurred with the high dosage of Esidrone should advise against the continued use of this drug clinically until electrocardiographic and other studies are performed to rule out serious cardiac embarrassment with the clinical dose now employed.

SUMMARY

In a series of trained, unanesthetized dogs the range of dosage between beginning tubular inhibition and the maximum obtainable chloride excretion varies for each mercurial diuretic. This range is suggested as a means of evaluating these diuretics. Within a range of dosage of 0.5 to 2.5 mgm. Hg/kgm., Salyrgan produced a maximum chloride excretion of approximately 200 mgm. NaCl/10 min./10 kgm. of dog. Esidrone (0.5 to 3.0 mgm. Hg/kgm.) produced a maximum excretion of 300 mgm. NaCl, and Mercupurin (0.5 to 6.0 mgm. Hg/kgm.) produced a maximum excretion of 500 mgm. NaCl.

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HEMOGLOBIN RADIOACTIVE IRON LIBERATED BY ERYTHROCYTE DESTRUCTION (ACETYLPHENYLHYDRAZINE) PROMPTLY REUTILIZED TO FORM NEW HEMOGLOBIN¹

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When large quantities of red blood cells are destroyed, as happens during crises of hemolytic icterus, malaria, sickle cell anemia, Bartonella infections, or as the result of drug administration (hydrazine and its derivatives), it is of interest to know the fate of the products of disintegration. In this paper we present data on the fate of iron liberated in such blood destruction produced in dogs by acetylphenylhydrazine. Such episodes are followed by a period of blood regeneration. In dogs with minimal iron storage, this probably means reutilization of the newly liberated iron. Another possibility, however, would be an irreversible deposition of this freed iron, together with blood regeneration utilizing iron taken from muscle hemoglobin and other tissue iron. In these experiments we have investigated this possibility by labeling the blood iron with the radioactive iron isotope.³ Such iron has been shown not to exchange physico-chemically with other iron of the body (6) and as long as the red cells remain intact, this tagged iron furnishes a means of following these cells throughout their physiological careers (3, 6).

METHODS. Routine care of these animals and preparation of diets has been described elsewhere in detail (9, 13). Hemoglobin was determined directly as oxyhemoglobin, using the photoelectric colorimeter with no. 54 green filter. Red blood cell counts were determined in the conventional manner. Measurements of radioactivity was done on a Geiger-Müller counter, using either a dipping type of counting tube as described by Bale, Haven and LeFevre (1), or a newly developed type of "inside counter" which will be described in a forthcoming publication. An effort was made to use as little blood as was consistent with accurate activity determinations in order that the circulating blood picture would not suffer by massive sampling. It was usually necessary to electroplate the iron under measurement and this procedure, which affords a decided increase in sensitivity of measurement, will be described elsewhere.

¹ We are indebted to Eli Lilly and Company for aid in conducting this work.

² On Official Commission from the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.

³ We are indebted to members of the Radiation Laboratory of the University of California and in particular to Drs. E. O. Lawrence and M. D. Kamen for the radioactive iron used in these experiments.

EXPERIMENTAL OBSERVATIONS. Dog 39-299 had been depleted of its iron reserve stores by repeated hemorrhage while being fed a diet low in this metal (9). At the beginning of iron feeding, the degree of microcytosis and hypochromia can be seen by the following figures RBC 4.4 M per cu. mm.; Hb 3.5 grams per 100 cc. blood, hematocrit 18 per cent; mean corpuscular volume 41 (cu. micra); mean corpuscular Hb. concentration 31 per cent; mean Hb concentration 8.0 (micro-micrograms). Iron containing the radio isotope was fed daily at a level of 30 mgm. over a period of 20 days. In figure 1 the parallel increase in hemoglobin and isotope concentrations of the whole blood are apparent. After administration was discontinued, both continued to rise for about a week, as might be ex-

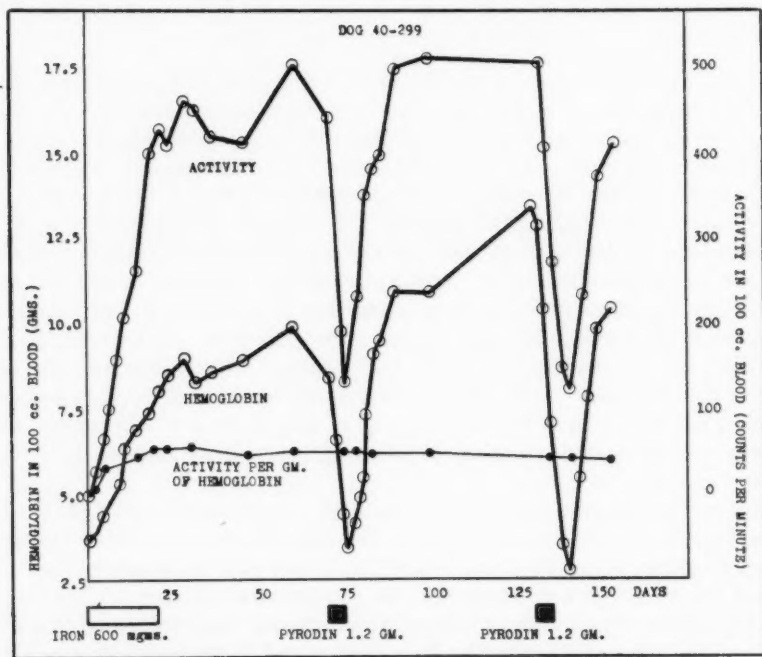


Fig. 1

pected (7), and then reached a constant, although subnormal, level. The mean corpuscular hemoglobin and mean corpuscular radioactivity likewise increased in parallel fashion during this iron feeding period. When the hemoglobin and activity concentrations had become relatively stable, acetylphenylhydrazine (pyrodin) was administered subcutaneously at a level of 300 mgm. per day for 4 days. The resultant drop in hemoglobin concentration and the parallel drop in activity concentration of the blood can readily be seen in figure 1. Following this during the regeneration phase, it will be noted that the activity concentration again parallels the hemoglobin concentration. The radioactivity per gram of hemoglobin did not change appreciably during this episode of blood destruction

and accompanying regeneration. Since experiments show that muscle hemoglobin and other tissue iron is not appreciably labeled with radioactive iron during an experiment of this type, this constancy of activity per gram hemoglobin can only mean, in view of the low iron stores, that the iron of the broken down hemoglobin has been rapidly reutilized during the regeneration process. The acuteness of this episode is shown by the hemoglobin level falling from 10 grams to 3 grams per 100 cc. of blood in the 6 days following the first pyrodin injection and a recovery to 11 grams per 100 cc. of blood 20 days after the first injection.

After establishment of a normal level of hemoglobin and a stable activity concentration, pyrodin was once more administered and the picture obtained before was again found.

Dog 40-213 was a normal animal when employed for some studies of susceptibility of red cells toward hypotonic salt solution (3). This dog was fed a diet of hospital table scraps and therefore the iron intake was not restricted.

In order to incorporate some radioactive iron into the circulating red cells, the animal was bled in all approximately 800 ml. Sixty-four milligrams of iron containing the isotope were administered intravenously and a sufficient period of time was allowed to elapse for the activity and hemoglobin concentration to become constant. At that time the blood picture was normal, RBC 5.5 M per cu. mm., Hb. 12.5 grams per 100 cc. blood. Hematocrit 42 per cent; mean corpuscular volume 76 cu. micra., mean Hb concentration 22.7 micromicrograms, mean corpuscular Hb. concentration 30 per cent.

Pyrodine was then administered and the resultant curves depicting concentration of hemoglobin and radioactivity can be seen in figure 2. It is possible that the reserve storage of ordinary iron in this animal had to a great extent been depleted by the bleedings referred to above, but in our experience repeated hemorrhage on a much greater scale is necessary to eliminate iron stores (9).

It will be noted again that the radioactivity per gram of hemoglobin remains essentially constant during the episode of blood destruction and recovery, indicating that the newly formed blood has iron of the same radioactivity as the broken down blood and that therefore this is probably the same iron.

To animal 39-320 iron was administered by mouth after it had been made anemic by bleeding. This iron was rapidly utilized in the formation of new blood cells. Following this regeneration of blood cells non-radioactive iron in the form of colloidal ferric hydroxide was administered by vein, including 200 mgm. after the hematocrit had reached the normal value. In this animal there is no question of reserve iron being available, since it has been shown that iron administered in this form can be used quantitatively in the production of hemoglobin in time of need (12). Table 1 shows the radioactive isotope concentration in the blood of this animal. During the period covered by these data, the red cells in circulation would presumably undergo breakdown due to ageing (4, 10). It is to be noted that the activity concentration is maintained at a relatively constant level throughout this period. If, following destruction due to natural physiological death, the "neutral" or storage iron had been used with equal readiness or in preference to the iron liberated from the red cells, we should ex-

pect a marked drop in the isotope concentration of the red blood cells due to dilution (4). This indicates that in blood regeneration under normal conditions, newly liberated iron is utilized even though ample storage iron is available.

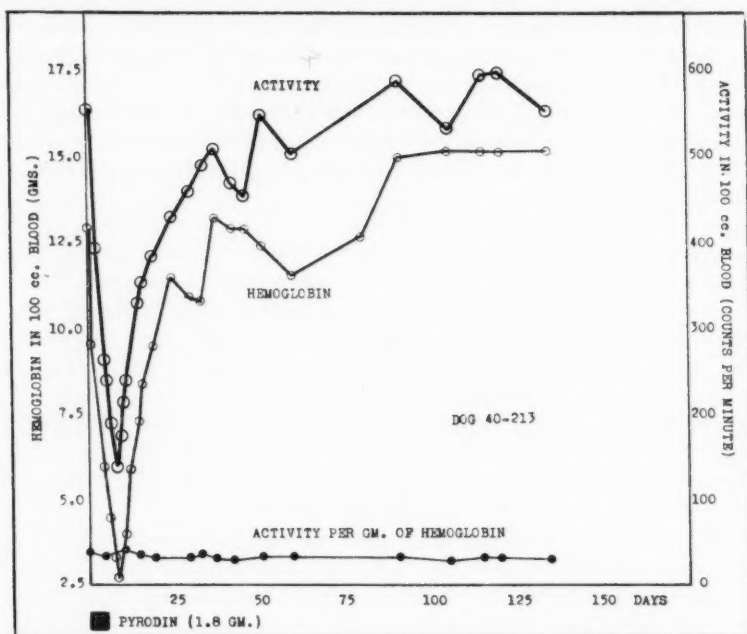


Fig. 2

TABLE 1
Dog 39-320

DAY OF EXPERIMENT	JUGULAR HEMATOCRIT	ISOTOPE CONCENTRATION	
		Red cells	Whole blood
	per cent	c./min.	c./min.
30	50.5	1600	810
60	50.0	1640	820
90	51.3	1730	888
120	48.2	1720	830
150	53.4	1735	927
180	52.5	1685	885

DISCUSSION. The fate of iron liberated during pathological destruction of blood elements, as well as during the normal turnover of blood cells, is of interest for several reasons. Is there a danger of depletion of the body iron as a result of repeated bouts of red cell destruction? Also the possibility of irreversible deposition of iron must be ruled out even though such a reaction needs hardly to be

anticipated. To answer the first of these questions it has been pointed out that excretion of iron under normal conditions is nearly negligible (5, 11), but that during blood destruction by acetylphenylhydrazine, a higher rate of excretion obtains, although this is still probably not of great consequence to the body economy (5, 8). Under the conditions of the present experiments the irreversible deposition of iron did not occur either during acute episodes of blood destruction or during the loss of iron due to the normal wear and tear of red cells. Instead, the newly liberated iron was used for the regeneration of new blood cells, probably even in preference to the normal storage iron present in the body.

As will be pointed out in a forthcoming paper (2), the blood picture shows morphological signs of blood regeneration as early as the fourth day after injection of pyrodin. This regeneration is very pronounced at the lowest level of hemoglobin; therefore, for instance in dog 40-299, the drop in hemoglobin in 100 cc. of blood from 9.5 to 3.3 does not represent the total blood destruction occurring. It seems likely that most of the red blood cells of the animal were destroyed and regenerated during each of these pyrodin episodes. Under these conditions the constancy of the radioactivity per gram of hemoglobin assumes special significance as an indication of the rapid reutilization of iron from the broken down red cells of the body, and also in eloquent witness for the speed by which iron incorporation into the red cell hemoglobin takes place.

SUMMARY

The iron liberated from hemoglobin derived from red cells destroyed by acetylphenylhydrazine (pyrodin) is utilized readily and nearly quantitatively for the regeneration of hemoglobin in the new red cells during the period of spontaneous recovery from anemia under the conditions of these experiments. Also during experiments with normal dogs, where ample reserve iron stores are available, it is found that the hemoglobin iron of new red blood cells is derived from the iron of old cells broken down in normal wear and tear of the animal blood, rather than from reserve stores.

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RADIOACTIVE IRON USED TO STUDY RED BLOOD CELLS OVER LONG PERIODS¹

THE CONSTANCY OF THE TOTAL BLOOD VOLUME IN THE DOG

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The radioactive isotope of iron² is useful not only in the study of iron metabolism (5) but also in studying the physiology of the erythrocyte (2, 3). By allowing an iron deficient, anemic dog to regenerate hemoglobin derived from a single oral administration of the radio iron, the age of the red cells into which the pigment is built can be considered as known to within a few days (3). The red cell into which the isotope has been introduced as a hemoglobin constituent is itself tagged very effectively. The other commonly employed artificial radioactive isotopes of such elements as phosphorus, sodium, potassium, chloride, etc., which occur alone as ions or in combination in the red cell, do so only transiently, either because of diffusion or due to a high rate of metabolic turnover. The tagged iron in the hemoglobin molecule is not subject to such vicarious changes. The hemoglobin of the red cell seems to remain intact as long as the cell exists (6, 9), and its component iron is not apparently subject to physico-chemical exchange in vitro (6).

Below are tabulated data to show that such exchange does not occur in vivo and that the red cell once tagged may be followed for many months. An obvious possibility would seem to be presented concerning the study of the life cycle of the erythrocyte directly. But the impracticability of this procedure is explained by the following experimental data.

METHODS. The animals used were normal, healthy, adult mongrels. They were all vaccinated against distemper and fed a diet of hospital table scraps containing adequate amounts of iron. In dog 39-320 the radioactive iron was administered orally but in dogs 39-242 and 38-137 the isotope was given by vein.

Blood volumes were performed at about weekly intervals, using a modification of the brilliant-vital-red dye method (11). Following establishment of a constant level of isotope and hemoglobin, iron in the form of non-radioactive colloidal ferric hydroxide was administered by vein. The amount supplied in this manner was of the order of magnitude of an animal's normal reserve stores (8).

¹ We are indebted to Eli Lilly and Company for aid in conducting this work.

² We are indebted to members of the Radiation Laboratory of the University of California and in particular to Drs. E. O. Lawrence and M. D. Kamen for the radioactive iron used in these experiments.

Dog 39-320 was a young adult female terrier, weighing 8 kgm. Three hundred fourteen milligrams of iron containing the radio isotope were given in the form of ferric ammonium citrate by gavage. The total activity was 70,000 counts per minute on a scale-of-four Geiger counter of the dipping variety (1). This animal had a circulating red cell volume of 260 ml. at normal hematocrit level, as determined by the radioactive donor cell procedure (7). Inert iron (312 mgm.) was given by vein as colloidal ferric hydroxide between the 10th and 17th days. One hundred ninety-two milligrams was also given on the 118th day (fig. 1).

Dog 39-137 was an adult female mongrel beagle, weighing 10 kgm. Seventy-one milligrams of the labeled iron was administered by vein in the form of ferric ammonium citrate. The total activity was 6,000 counts per minute. The circulating red cell volume was 272 ml. at normal hematocrit level (7).

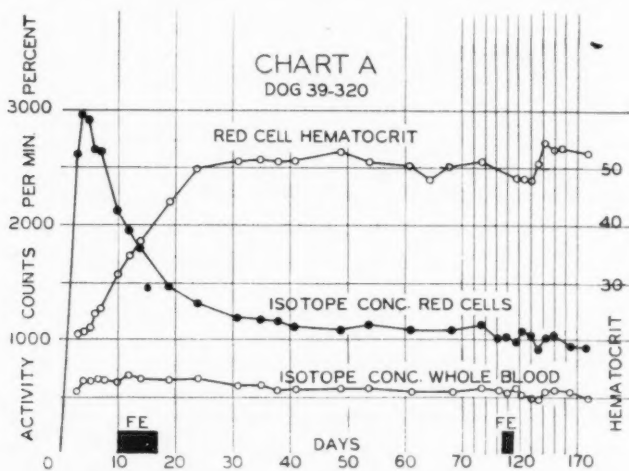


Fig. 1

Inert iron (232 mgm.) was given by vein as colloidal ferric hydroxide between the 50th and 60th experimental days (fig. 2).

Dog 39-242 was a 11.5 kgm. male adult mongrel terrier. Thirty-one milligrams of the labeled iron was administered as ferric ammonium citrate by vein. The red cell circulating volume was 450 ml. determined at normal hematocrit level (7). Inert iron (180 mgm.) was given by vein as colloidal ferric hydroxide near the 75th experimental day (fig. 3).

Activity measurements were made on a Geiger-Müller counter, using a dipping type of counting tube in the early parts of these experiments.

Later, when the radioactivity became too low for measurement with this apparatus, the iron was electroplated on to tin foil and measured on a counter with thin aluminum walls. Due to lower absorption of iron beta rays by this counter's walls, a sensitivity increase of thirty times is obtained.

The counter operates at 7 cm. hydrogen and 1.0 cm. alcohol pressure. In order that the thin walls will not collapse from external pressure, the whole counter including the iron sample is contained in an evacuated brass cylinder. A double turret mechanism operated from outside allows four samples to be measured before the pressure is raised to atmospheric and the samples changed.

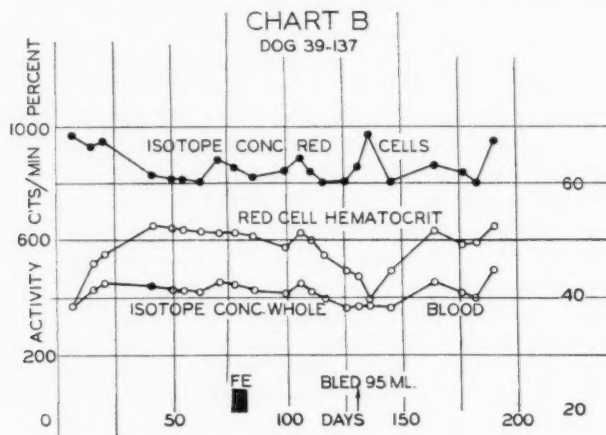


Fig. 2

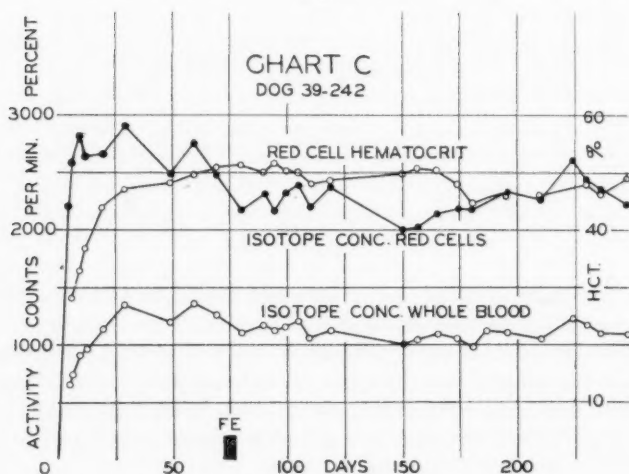


Fig. 3

The counter walls are of 0.001 cm. aluminum with a thin layer of copper evaporated on the interior for better counter characteristics. The sensitive area is 1.2 cm. diameter by 5 cm. long. Where the 3 cm. long iron sample is in position to count it is concentric with and about 1 mm. from the counter wall. This counter level has a low background count of 3.5 scale-of-four counts per minute

and the same good sensitivity for radio iron wherever it may be located on the plated sample.

For electroplating³ the ferric hydroxide is dissolved in a few drops of hydrochloric acid and evaporated to near dryness. The ferric chloride is transferred with a few milliliters of water to the electrolytic cell and 3 ml. of 20 per cent ammonium chloride and 2 ml. of saturated sodium citrate added. The whole is made up with water to 15 ml. The anode is a platinum wire and the cathode a cylinder of tin foil 1 cm. in diameter by 3 cm. long, fitting closely the wall of the electrolytic chamber, with an attached tab for handling and electrical connections. The cylinder is coated on the outside with red Glyptal⁴ and the tab is completely covered, except at the end where electrical connection is made. The solution is stirred by a current of air and immersion in a tank of rapidly moving water to provide cooling. The plating is carried out for 4 hours at about 1.5 amperes provided by a 200 watt lamp in series with the D.C. supply. At the end of the plating, the electrolyte is acidified with hydrochloric acid and tested with ammonium thiocyanate to be sure no iron remains. Each solution is made up to contain 3 to 5 mgm. of iron by adding inert iron to solutions known to be low in total iron content.

The corrected concentrations of isotope levels were determined as follows. The determined concentration in red cells was multiplied by the red cell volume to provide a value for total circulating isotope. The amount of isotope activity removed previous to this time by sampling procedures was added to this value. Division by the red cell volume then provided the concentration of isotope in red cells corrected for sampling. The whole blood corrected concentrations were then obtained by multiplying corrected red cell isotope concentrations by the jugular hematocrit present in each case.

These corrections are accumulative and toward the end of the experiments may approach 60 per cent of the total count. They are, of course, much smaller than this during most of the experimental period.

DISCUSSION. Examination of figures 1, 2 and 3 discloses that the isotope concentration of the erythrocytes is remarkably constant over a very long period. If exchange of iron in the red cell hemoglobin with body stores or other iron takes place, it is of a negligible extent, since ample amounts of ordinary iron are available for such exchange in these animals, it having been introduced by vein in each case.

Hawkins and Whipple (9) have shown that the life of the red cell is approximately 115 to 130 days in the bile fistula dog. They utilized the quantitative liberation of bile pigment from aging red cells for their demonstration. In animals whose circulating cells were tagged with a given amount of isotopic iron, the concentration of activity in the circulation supposedly would remain constant until disintegration of the tagged cells. Presumably, the new cells then formed would derive their iron partly from the inert storage iron and partly from

³ We should like to acknowledge the assistance of Hoyt Whipple in development of the electroplating procedure.

⁴ Obtained from General Electric Company.

the disintegrated erythrocyte hemoglobin. This would result in a telltale dilution of the circulating activity and should occur at the end of the life cycle of the original tagged cells. In the animals under consideration, storage iron was adequate and it has been shown that this storage iron is utilized very readily for hemoglobin construction in anemia due to blood loss (8). Nevertheless, there was no change in the concentration of the red cell or whole blood isotope level during the time of expected life cycle breakdown of about 120 days. From this we can infer that either in the normal unoperated dog (intact biliary system) the red cell life cycle is greater than in the operated dog or, as is more likely, the iron liberated from the hemoglobin of the destroyed red cells is more promptly utilized than iron in storage. Dietary iron is probably not of significance in these experiments since in all likelihood, under these conditions, very little iron would have been absorbed (5).

It might be mentioned to advantage also that if a considerable number of red cells were undergoing disintegration by circulatory trauma (daily wear and tear), and the iron liberated from these cells was likewise handled preferentially in the construction of new cells, it would not be possible to use the isotope in the determination of this wear and tear factor.

The red cell hematocrit changed over a wide range following complete utilization as shown by peak in the concentration of the isotope (fig. 1). The concentration of radioactive iron in the red cells appears to change as an indirect function of this hematocrit. This suggests a mathematical product of the corresponding values which would be constant. If the latter procedure is carried out, a nearly straight line results when the product of red cell isotope concentration and jugular hematocrit is plotted against time. We may consider why this occurs.

It has been stated that the isotope when incorporated into the red cell as hemoglobin, remains there for a considerable time (3). This is also apparent from inspection of the figures 1, 2 and 3 below. Therefore, if there is no exchange of the isotope with other iron of the body (6) or loss by excretion (4) within the life span of the containing red cell, we may say that the total amount of circulating isotope remains relatively constant after the supply of it has been exhausted by use. This may be expressed as follows:

$$\text{Total RBC mass} \times \text{conc. of isotope in RBC's} = K_1$$

Since it has been shown that the *circulating* red cell mass in the dog is approximately equal to the *total* red cell mass (7) and since it has also been shown that the latter value is about 75 per cent of the value derived indirectly from determination of plasma volume in the dye procedures (10) (7).

$$(A) \quad \text{Blood vol. (dye)} \times \text{Jug. Hct.} \times 0.75 \times \text{conc. RaFe in RBC's} = K_2$$

and according to the experimentally determined relationship mentioned above:

$$(B) \quad \text{Conc. of RaFe in RBC's} \times \text{Jug. Hct.} = K_3$$

by dividing A by B it follows that the simple relationship,

$$(C) \quad \text{Blood vol.} = K_4$$

exists even when there is a marked change in jugular hematocrit (fig. 1).

The same conclusion may be reached if values for the concentration of isotope in the whole blood plotted against time are compared to values of the jugular hematocrit and the concentration of isotope in the red cells (fig. 1). The concentration of the radio iron in the whole blood remains essentially constant in spite of a rise in the jugular hematocrit and a corresponding fall in the concentration of the radio iron in the red cells, the latter due to dilution by cells containing ordinary iron from the diet or other extraneous sources. This also indicates that the total blood volume is a constant. Therefore, as the volume of red cells increases, there would seem to be a loss of plasma from the circulation to permit such a picture, and conversely, when there is a loss of red cells from the circulation one would expect a comparable increase in plasma volume.

SUMMARY

When the circulating erythrocytes have been tagged by the incorporation of radioactive iron into their constituent hemoglobin, these cells may be followed in the body for many months.

When disintegration of the red cells occurs, either by aging or trauma, even in the presence of adequate inert storage iron, the labeled iron from the liberated hemoglobin is almost immediately re-utilized by new cells such that the total circulating radioactivity is maintained constant.

It is not feasible in these experiments to use the iron isotope in the determination of the life cycle of the red blood cell.

It is indicated from these experiments that the total blood volume of the dog is maintained at a constant level independent of the state of anemia. As the red cell circulating volume increases, there is a corresponding drop in the plasma volume in order to maintain the total circulating blood volume constant.

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THE EFFECT OF PARTIAL HEPATECTOMY ON THE BLOOD VOLUME IN THE WHITE RAT

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In their review of the literature concerning the plasma proteins, Madden and Whipple (1) referred to the depression of the plasma proteins which occurs in rats within twenty-four hours after partial hepatectomy, and stated, "It would be interesting to know something about blood volume at this time in these rats." This work is concerned with the determination of blood volumes at varying periods after partial hepatectomy in the white rat.

METHODS. White male rats of Wistar strain, four to six months of age, raised on a stock diet, were used. Partial hepatectomy, under ether anesthesia, was performed according to the procedure of Higgins and Anderson (2). Intact animals of the same age served as controls. After operation the animals were continued on the stock diet, and were allowed food and water *ad libitum*. Blood volume determinations were made with the dye method of Gibson and Evans (3) and Gibson and Evelyn (4), as modified for rats by Beckwith and Chanutin (5). For this procedure the animals were anesthetized with intraperitoneal sodium-pentobarbital, with precautions outlined by Sheffley and Higgins (6). In a few instances two blood volume determinations were made on the same rat at different periods after partial hepatectomy, but in most cases the animals were sacrificed after one determination.

RESULTS. Data for total blood volume, plasma and red cell volumes are presented graphically in figure 1. Plasma and red cell volumes decreased comparably during the first days, and as a result none of the values for total blood volume fell within the control range during this period. Thereafter the plasma volume rose progressively, reaching the average control value on the seventh day; the individual and average values for plasma volume continued to increase appreciably and in many cases were greater than those of the control group. The majority of values for red cell volume were within the control range on the seventh day, but the average value had not reached the control level on the twentieth day. The return of the total blood volumes to the control level about the ninth day was due principally to the increased plasma volumes.

Blood volume determinations on animals subjected to simple laparotomy alone showed no deviation from the control range on the first and third days after operation.

COMMENT. It has been observed (7) that following cessation of a moderate hemorrhage, the blood volume is quickly restored to its previous level, presum-

ably by absorption of extravascular fluid; and that the percentage of red cells and hemoglobin is further decreased by this dilution. In the present experiment the decreased blood volume was due to diminution in both the plasma volume and the red cell volume, but since the plasma volume did not increase until five days after operation, at which time the red cell volume also rose slightly, the blood volume changes do not appear to be the result of simple hemorrhage. Moreover, the alteration of blood volume seems greater than can be accounted for by blood loss incident to operation and removal of liver tissue.

The concentration of the plasma albumin may be one factor in the regulation of the plasma volume (8), but has been found to be significant only when the red cell volume is constant (9). Chanutin and associates (10) found that the con-

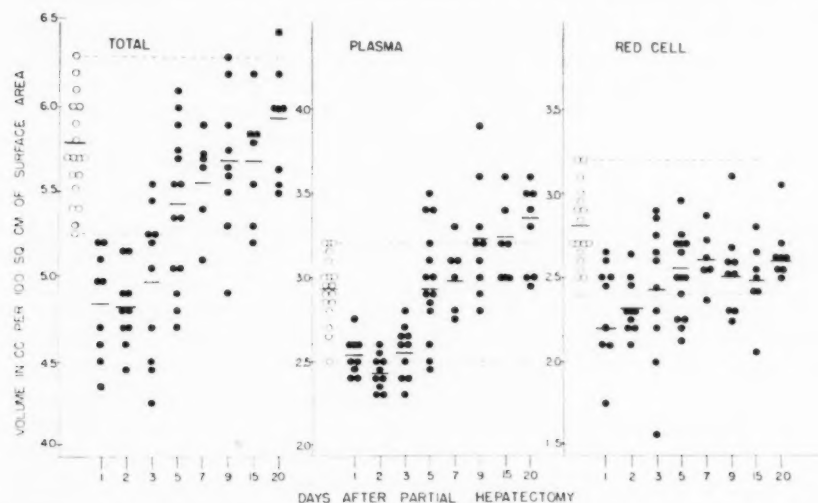


Fig. 1. Changes in the total, plasma and red cell volume after partial hepatectomy. Open circles represent intact, control animals. The dotted lines designate the minimum and maximum variations for the control rats.

centration of the plasma albumin is decreased within twenty-four hours after partial hepatectomy, and that it tended to remain low until after the eighteenth day. Similarly, values for total protein were depressed within twenty-four hours, but returned to the control range on the fifth day. From the present observations, it would appear that the plasma volume and the total blood volume may return to control levels even though the plasma albumin concentration remains depressed.

SUMMARY

Plasma, red cell and total blood volumes were determined in partially hepatectomized rats, at frequent intervals after operation. During the first forty-eight hours each of these was decreased markedly. The plasma volume reached the

control level on the seventh day after operation; total blood volume on the ninth day; and the majority of red cell volume determinations were within the control range on the seventh day.

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GASTRIC INHIBITION CAUSED BY AMINO ACIDS IN THE SMALL INTESTINE

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In a previous report Thomas and Crider (1939) called attention to the fact that various products of protein digestion, including some of the amino acids, cause inhibition of gastric peristalsis when placed in the small intestine. Some details regarding the action of the amino acids were reported separately (Thomas, 1939) and a further study was promised. Up to the present sixteen amino acids and some amino acid mixtures have been investigated on ten dogs.

METHODS. The arrangements for recording gastric (antral) peristalsis in dogs provided with permanent gastric and duodenal fistulas, and for injecting test solutions into the small intestine were described in the communications just mentioned. As in the previous study, experiments were performed both before and after feeding and with continuous drainage of the first part of the duodenum to prevent contamination of the injected material. The results were qualitatively the same whether the stomach was full or empty but the responses of the full stomach to inhibitory stimuli were less pronounced and of shorter duration than those of the empty stomach.

The amino acids were obtained from various commercial sources and were of different degrees of purity but enough pure preparations were available to prove that the results were not noticeably affected by the impurities encountered. With a few exceptions amino acids were administered in 2 per cent solution. This concentration was selected as a satisfactory compromise between the requirements of isotonicity on the one hand, and the need for a uniform concentration for purposes of comparison, on the other. The acids of low molecular weight are hypertonic in 2 per cent solution but since they had little effect anyway no error was introduced. Those acids that are not soluble to the extent of 2 per cent were either prepared in supersaturated solution with the aid of heat or used in lower concentration, with the exception of tyrosine and cystine which were suspended in normal saline.

Usually 20 cc. of the preparation were injected into the lumen of the intestine to determine the effect on gastric peristalsis. However, larger amounts were used when relatively inactive material or a resistant animal was encountered.

RESULTS. *The monoamino-monocarboxy acids.* All the members of this relatively large group of amino acids caused some degree of gastric inhibition when injected into the small intestine in neutral solution. However, the effect was so slight with some of them, e.g., glycine and serine, that it could be demonstrated

only under the most favorable conditions and can, therefore, have little physiological significance.

In general the inhibitory effect was greater the greater the molecular weight of the amino acid used. Particular attention was, therefore, given to the amino acids of the leucine series in which the molecular weights are identical. Leucine and norleucine were about equally effective. Isoleucine was less active and often failed to cause gastric inhibition. Norleucine frequently had a peculiar diphasic effect consisting of prompt primary inhibition followed by partial recovery and secondary inhibition reaching its maximum about five minutes after injection.

The amino acids having the most pronounced inhibitory effect were the aromatic acids, phenylalanine, tyrosine and tryptophane. Tryptophane in particular caused pronounced and prolonged gastric inhibition even in 1 per cent solution (fig. 1, upper graph). Tyrosine caused good inhibition when injected in suspension in 0.9 per cent sodium chloride. Since the solubility of tyrosine in cold water is only about 0.04 per cent, it is evidently a fairly potent inhibitory agent. Doubtless it is somewhat more soluble in the intestinal juices.

Following is a list of all the amino acids in this series with their molecular weights, which have been investigated, arranged in the order of increasing gastro-inhibitory action:

<i>Amino Acid</i>	<i>Mol. Wt.</i>	<i>Amino Acid</i>	<i>Mol. Wt.</i>
Glycine.....	75.05	Leucine.....	131.11
Serine.....	105.06	Norleucine.....	131.11
Alanine.....	89.06	Phenylalanine.....	165.09
Valine.....	117.09	Tyrosine.....	181.09
Isoleucine.....	131.11	Tryptophane.....	204.11

The monoamino dicarboxy acids. The gastro-inhibitory activity of glutamic and aspartic acids was, apparently, governed by the pH of the solution in which they were administered. When injected as free acids (pH 3.2-2.9) they caused more complete and prolonged gastric inhibition than any of the other amino acids used except tryptophane and, possibly, phenylalanine. When the pH was progressively increased by addition of increasing amounts of NaOH, their inhibitory effect was progressively diminished; it was still easily demonstrated at pH 4.0 but was practically absent at pH 5.0. At pH 7.0 their solutions had no more inhibitory effect than an equal volume of normal saline.

In the hope of determining whether these substances, when effective, were acting as amino acids or merely as sources of hydrogen ions, their effect on gastric motility was compared with that of isotonic phosphate buffers and lactate buffers at various pH levels. The phosphates caused no gastric inhibition above pH 3.0; the lactates, on the other hand behaved like the salts of aspartic or glutamic acid although their action was weaker.

One peculiar observation which was repeated several times suggests that the inhibitory effect of these acids as well as that exhibited in less degree by the diamino acids (see following section) may have been due to an excess of hydrogen or hydroxyl ions respectively. A solution of free glutamic acid at pH 3.2 was added to a mixture of diamino acids in solution at pH 9.5 until the resulting mix-

ture had a pH of 7.0. The neutral mixture was without effect on gastric motility although the original acid and alkaline solutions both caused inhibition.

If we wish to assume that the gastro-inhibitory effect of the dicarboxy amino acids and other organic acids is due to hydrogen ions the fact that they are effec-

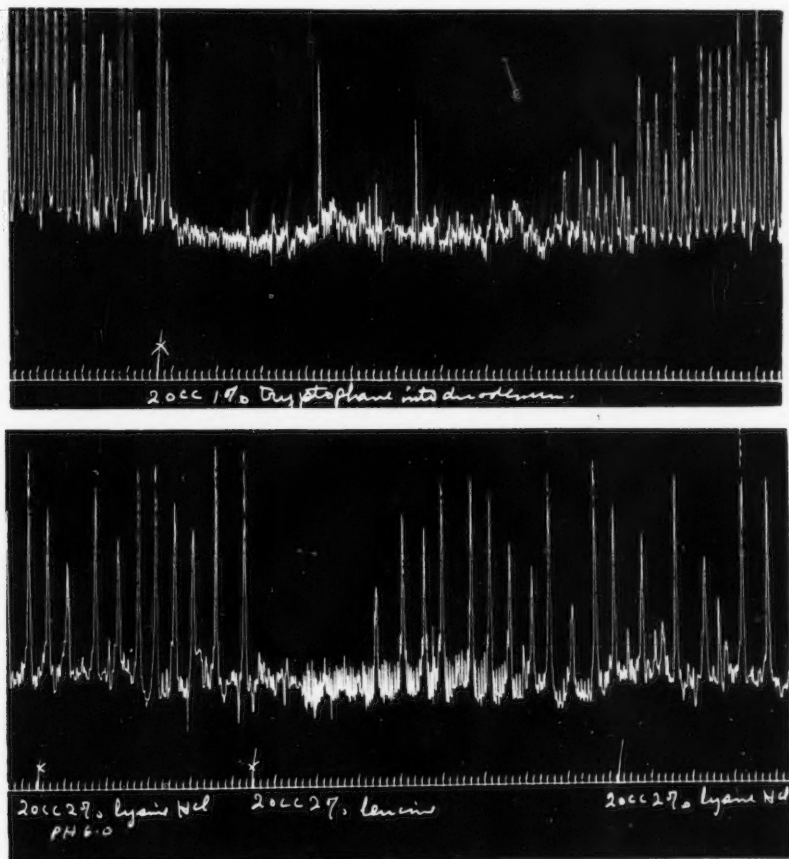


Fig. 1. Upper graph: Effect on gastric peristalsis of injecting 20 cc. of 1 per cent tryptophane solution at pH 5.9 into the duodenum.

Lower record: Negative results with 2 injections of 20 cc. each of 2 per cent lysine HCl at pH 6.0. One injection preceded and the other followed an injection of 20 cc. 2 per cent leucine at the same pH, used for comparison.

The records were made with a water manometer. Time is in ten second intervals.

five at pH levels at which inorganic acids fail to act may be explained by the superior buffering efficiency of the organic acids and their salts. This possibility has been discussed previously in connection with a different problem (Thomas and Crider, 1940).

The diamino acids. Arginine HCl and lysine HCl (fig. 1, lower graph) were studied; also a mixture of diamino acids, presumably including histidine, precipitated from an acid casein digest by means of phosphotungstic acid. All these preparations behaved in a parallel manner. When carefully neutralized they had little or no inhibitory effect on gastric peristalsis. The free "acids" (pH 9.0 +) caused some inhibition and in some instances slight nausea. Arginine HCl, which is strongly acid in reaction, also caused slight gastric inhibition. There was nothing in the results to suggest that these substances contribute in any substantial degree to the gastric inhibition caused by neutral amino acid mixtures.

Other amino acids. Cystine caused no gastric inhibition. This is not surprising in view of its almost complete insolubility in water. Efforts to prepare supersaturated solutions by quick neutralization of alkaline solutions were only partly successful and failed to yield evidence of an inhibitory action. Proline and hydroxyproline regularly caused moderate gastric inhibition, corresponding approximately to that caused by alanine.

Optical activity. Most of the amino acids used were racemic. Exceptions were *l*-leucine, *l*-tyrosine, *l*-cystine, *l*-tryptophane, *l*-aspartic acid and the glutamic acid, some of which was marked "*p*" and some "*d*." It is not clear whether the latter were actually different in optical activity or were merely labeled according to different concepts. Direct comparisons were not made between laevo- and dextrorotatory samples of the same amino acid, and for that reason the possibility that optical activity is a factor in determining the gastro-inhibitory effect was not excluded. The fact that the inhibitory activity showed a consistent relation to molecular weight in some instances and to pH in others in spite of the haphazard distribution of optical activity suggests, but does not prove, that the latter did not influence the results.

DISCUSSION. In view of the consistent gastro-inhibitory effect of a majority of the amino acids studied the failure of the dicarboxy and diamino acids to cause inhibition in neutral solution is surprising. Two possible explanations are suggested. It may be that all the amino acids cause gastric inhibition when in the free state, that is, uncombined with acid or base, but are ineffective when combined as the hydrochloride or the sodium salt. The acids that are effective in neutral solution all form solutions that are nearly neutral to begin with and only a small part of the total acid need be combined to fully neutralize them. The ineffective acids, on the other hand, form strongly acid or alkaline solutions and are almost completely combined with base or acid in neutralized solutions. The other possibility is that the amino acids that are ineffective in neutral solution possess no gastro-inhibitory properties and owe their effectiveness in the free state to the acidity or alkalinity of their solutions. The experiments do not indicate which explanation is correct.

Whether the gastro-inhibitory action of the amino acids contributes materially to the regulation of gastric motility under normal circumstances is doubtful. Any effect they might have would depend on the attainment of an adequate concentration in the intestine. The intestinal contents are generally isotonic and

they contain, besides products of digestion, other osmotically active substances, for example, inorganic electrolytes. The amount of amino acids and other end products of digestion which can accumulate without causing hypertonicity must be relatively small. Probably they are absorbed about as fast as they are produced. For this reason the inhibitory effect of the amino acids is probably not an indication of any special adaptation of the reacting mechanism to these substances as such. It is more likely incidental to the fact that they exhibit properties similar to the more abundant, and therefore more effective, intermediate products of protein digestion. Study of the amino acids may enable us to identify some of these properties, and in this way to increase our understanding of the inhibitory action of the proteoses and peptones.

SUMMARY

1. Sixteen amino acids were studied to determine whether they caused gastric inhibition when placed in the small intestine of unanesthetized, fistula dogs.

2. Only the monoamino-monocarboxy acids caused gastric inhibition regularly when administered in neutral solution. The inhibitory effect of these acids was roughly proportional to their molecular weights but the evidence indicates that it was also influenced by other factors.

3. The dicarboxy acids and the diamino acids caused gastric inhibition when administered as free acids without neutralization but were ineffective in neutral solution.

4. Experiments designed to determine whether the dicarboxy and diamino acids owe their gastro-inhibitory activity to acidity and alkalinity, respectively, or to their amino acid structure, were inconclusive.

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RESPONSE TO GROWTH HORMONE OF HYPOPHYSECTOMIZED RATS WHEN RESTRICTED TO FOOD INTAKE OF CONTROLS¹

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Lee and Schaffer observed that young normal rats, when treated with growth promoting extracts from the anterior pituitary, gained significantly more weight than did untreated littermates, though food intakes were kept identical for both experimental and control animals (1, 2). From these experiments they concluded that the pituitary gland promotes growth by causing deposition of tissue substance through better utilization of the consumed food, and not only as a consequence of an increased food intake.

Since the growth promoting extracts available at the time were not free of pituitary "target organ" hormones, the better utilization of the consumed food and the resulting increased growth might have been due to contaminations and not to the growth hormone itself. Furthermore, the very existence of a specific growth promoting substance has been questioned (3, 4). Therefore, it seemed to be highly important to repeat the work of Lee and Schaffer with a growth hormone preparation free as far as possible of other hormones. This was rendered possible through recent advances made in the purification of the growth hormone. Preparations can be obtained now which are high in growth promoting activity, but, at the same time, practically free of "target organ" hormones (5).

Furthermore, normal rats were used by Lee and Schaffer, so that the observed effect might have occurred through an action, direct or indirect, of the extract on the animal's pituitary. Because it seemed important to exclude these possibilities, the pituitary glands of the test animals were removed in the present work previous to the experimental period. The following paper reports the result of the injection of a purified growth hormone preparation into hypophysectomized rats restricted to the food intake of untreated hypophysectomized controls.

Two experiments were carried out similarly in every respect except for the method of feeding. Female immature hypophysectomized rats were used, operated upon at 26 to 28 days of age, after a post-operative period of 11 to 13 days. Their average body weights were 65 and 69 grams respectively at onset of the injection period. The animals were divided in 2 groups, one receiving

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daily intraperitoneal injections of a growth hormone solution, the other saline as control. The growth hormone preparations used were cysteine-treated globulin fractions (5, 6), containing approximately 25 and 50 growth hormone units per milligram (7), respectively, when standardized in hypophysectomized rats (10 day test). They were found practically free of other "contaminating" pituitary ("target organ") hormones when assayed at relatively high levels: Lactogenic hormone was not demonstrable at a total dose of 20 mgm. in month old pigeons (systemic crop test, daily subcutaneous injections for 4 days). The follicle-stimulating, interstitial-cell-stimulating and adrenocorticotrophic hormones were not demonstrable at total doses of 4.0 and 4.5 mgm., respectively, in hypophysectomized rats (4 and 10 day tests, based on histological evidence for repair of ovaries and adrenals). At levels of 5.0 and 10.0 mgm., respectively, thyrotropic hormone was not demonstrable in month old squabs (4 day test based on histological evidence for repair of thyroids). Furthermore, in the hypophysectomized rat, a more sensitive test object, thyroid stimulation was evident neither at the histological examination of the thyroids in experiment II of the present series, nor in another assay of the same growth hormone preparation at a higher dose level (4.5 mgm., 10 day test). In experiment I the smallest amount of histologically detectable thyroid response occurred. This represents an extremely small contamination of the growth hormone preparation with thyrotropic hormone, approximately one per cent by weight, a quantity of thyrotropic hormone which is far below the amount necessary for functional thyroid stimulation.

The food consumption was controlled so that, in each case, the food intake of hormone-treated and control rats was identical during the experimental period. In experiment I a measured amount of food was given twice daily by stomach tube and, in addition, a measured amount over night in the food cups. In the second experiment Mitchell's paired feeding method was employed (8). The control animals were permitted to eat *ad libitum*, and the average food consumption was determined for each day. The treatment of the experimental rats was started 2 days later, these animals receiving on each day the same amount of food as determined for the controls 2 days earlier. All rats were kept in single cages. A modification of McCollum's "diet I" was given. In order to permit passage of the diet through the narrow catheter tubing used as stomach tube, whole wheat was substituted by wheat flour, and this modified diet was fortified by a vitamin B concentrate.²

The resultant body weights (table 1) indicate that there was, in spite of an identical food intake, a remarkable increase in body weight in the hormone-treated rats, as against practical growth stasis in the control groups. It is obvious that in these experiments the growth-promoting action of the hormone cannot be ascribed to increased food intake. The resulting deposition of tissue substance must therefore be considered as a consequence of a better utilization of the food eaten.

² Composition of McCollum's "diet I," modified: Casein, 20 per cent; white flour, 52.5 per cent; whole milk powder, 10 per cent; calcium carbonate, 1.5 per cent; sodium chloride, 1 per cent; vitamin B concentrate ("Galen B"), 10 per cent; butter, 5 per cent.

In order to investigate whether purified growth hormone has any effect on organ weights, autopsies were performed on all rats at termination of the injection period.³ The results of both experiments are summarized in table 2. All organs were found heavier in the group of rats treated with growth hormone.

TABLE 1

Effect of pituitary growth hormone on body weight of hypophysectomized rats restricted to food intake of untreated controls

Duration of experiments: 10 days

EXPERIMENT NUMBER	NUMBER AND GROUP OF RATS	TOTAL DOSE PER RAT	AVERAGE BODY WEIGHT GAIN	
			Grams*	Per cent of body weight
I	8 injected	2.4	16 (10 to 20)	25
	9 controls	0	2 (-2 to 6)	3
II	9 injected	2.5	15 (13 to 17)	23
	10 controls	0	0 (-4 to 7)	0

* Figures in brackets indicate the range of body weight gains.

TABLE 2

Effect of pituitary growth hormone on organ weights of hypophysectomized rats restricted to food intake of untreated controls

Summary of two experiments; duration: 10 and 11 days, respectively

NUMBER AND GROUP OF RATS	OVARIES	ADRENALS	THYROID	THYMUS	SPLEEN	CLN*	LIVER	KIDNEYS	STOMACH	INTESTINE
Organ weights in milligrams										
17 injected.....	9.9	10.8	9.6	249	302	102	3,260	814	613	3,980
19 controls.....	8.0	8.0	6.2	134	194	93	2,290	664	474	3,090
Organ weights as per cent of body weight										
17 injected.....	0.0123	0.0133	0.0120	0.307	0.376	0.125	4.04	1.009	0.758	4.92
19 controls.....	0.0116	0.0116	0.0091	0.196	0.280	0.127	4.34	0.966	0.692	4.49

* Cervical lymph nodes: One experiment only; 8 injected and 7 control rats.

However, the organs did not all grow at the same rate as the whole body. Since the difference in body weight between experimental and control rats at time of autopsy was almost 25 per cent, the values were also expressed in per cent of body

³ Ovaries, adrenals and thyroids were examined histologically, in order to check the purity of the growth hormone preparations used.

weight (table 2). In all instances where the differences between these percentage values for experimentals and controls (D) exceeded 6 per cent, standard errors (S_D) were calculated for those differences (table 3). A difference was considered statistically significant when the value $D/S_D > 3$ (9).

TABLE 3

Effect of pituitary growth hormone on organ weights of hypophysectomized rats restricted to food intake of untreated controls

Statistical significance of difference between organ weights of injected and of control rats, expressed in per cent of body weight

	ADRENALS	THYROIDS	THYMUS	SPLEEN	LIVER	STOMACH	INTESTINE
Difference (D).....	0.0017	0.0029	0.111	0.096	0.30	0.066	0.43
Standard error (S_D).....	0.0010	0.0009	0.016	0.036	0.13	0.034	0.14
D S_D	1.7	3.2	6.9	2.7	2.3	1.9	3.1

It is obvious that, through the action of the growth hormone, the thymus was increased proportionately more than any other organ and more than the body as a whole. This may indicate a specific thymotropic effect of the growth hormone preparation employed. This effect is the more remarkable since it has been shown that the thymus is not required for the growth of normal animals nor for the body weight response of either normal or hypophysectomized animals to administered growth hormone (10). The other organs, as far as studied, grew approximately at the same rate as the whole body (ovaries, kidneys, cervical lymph nodes), or at a slightly higher rate. The values for the difference, however, were statistically significant only for thyroids and intestine, not significant for adrenals, spleen and stomach. The only organ that showed a decrease in weight (expressed as per cent of body weight), though not statistically significant, was the liver. This trend is in agreement with recent observations (11).

SUMMARY

When care was taken to secure an identical food intake, hypophysectomized rats treated with a purified growth hormone preparation from the anterior pituitary gained significantly more weight than their untreated controls. This would indicate that the growth hormone caused increased deposition of tissue substance, not as a consequence of increased food intake, but through better utilization of the consumed food.

All internal organs examined were heavier in the groups treated with growth hormone. They grew only approximately at the same rate as did the body as a whole, with exception of the thymus, which grew considerably faster.

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NERVOUS PATHWAYS FOR THE REFLEX REGULATION OF INTESTINAL PRESSURE¹

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The motor responses of the gastro-intestinal tract to stimuli arising from alterations of pressure within it are modulated by long visceral reflexes. Distention of a jejunal segment in the form of a Thiry fistula in unanesthetized dogs results in inhibition of gastric tonus and motility (6). Acute experiments on chloralosed dogs have shown that distention of the intestine results in immediate inhibition of other segments of intestine having no intrinsic connections with the distended segment (2). In similar experiments (7) it was found that the stomach showed decreased tonus and motility as a result of intestinal distention. These so-called intestino-gastric and intestino-intestinal inhibitory reflexes were, according to Morin and Vial (8), abolished by bilateral splanchnicotomy; but they were not noticeably affected by bilateral vagotomy. Therefore, it was concluded that the reflexes were mediated entirely through the splanchnic nerves. However, Lalic et al. (6) found that the intestino-gastric inhibitory reflex in unanesthetized dogs may be mediated by either sympathetic or vagal pathways. It is possible that the anesthetic drug used in the experiments of Morin and Vial (8) may have interfered with a vagal intestino-gastric inhibitory reflex. Moreover, failure to obtain the reflex after one set of pathways is destroyed does not preclude the possibility that the other set of nerves contains one limb of the reflex arc.

In confirmation of the earlier studies on anesthetized dogs (2), Youmans, Meek and Herrin (14) found that distention of either of two intestinal segments in the form of Thiry fistulae in unanesthetized dogs results in immediate nervous inhibition of the other segment. The present study is concerned with the investigation of the rôle of vagal, sympathetic, and pre-aortic nervous pathways in the intestino-intestinal inhibitory reflex in unanesthetized dogs. Attention has also been given to the effect of the various denervations on the pain response to distention.

METHODS. Nine dogs were prepared each having two Thiry fistulae made from adjacent segments of the upper jejunum. After determining for each dog, by recording methods identical to those previously described (14), that inhibition of one segment of intestine could be obtained by distention of the other segment the dogs were subjected to aseptic operations as follows. Three dogs were bilaterally vagotomized at the level of the lower esophagus, and a fourth was bi-

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laterally vagotomized in the mid-cervical region. The splanchnic nerves were cut, and the lumbar sympathetic chains were removed from the other five dogs. Some details concerning the methods used in the latter operations are described under section II of the results. After recovery from the operations the effect of distention of one segment on the motility of the other segment was determined again in each dog. The local response to distention was also determined in segments having only vagal or only sympathetic innervation for comparison with the local responses to distention in completely denervated and in normal segments (10).

RESULTS. The observations described under sections I through III below are based on over one hundred separate distentions in the nine dogs. The effect of distention of one intestinal segment on the motility of another segment when both sets of extrinsic nerves are intact has been described (14). The immediate and, usually, complete inhibition of the undistended segment which is obtained under these conditions will, following the practice of Morin and Vial (7), be called the intestino-intestinal inhibitory reflex. Procedures which abolish this reflex do not necessarily prevent the appearance of delayed inhibitory effects as a result of prolonged intestinal distention. However, these latter effects are on a chemical or humoral basis and are not under consideration in this study.

I. *Effect of vagotomy on the intestino-intestinal inhibitory reflex.* Each of the four bilaterally vagotomized dogs showed inhibition of either intestinal segment during distention of the other segment. The sensitivity was undiminished by the vagotomy, and the onset of the inhibition was just as rapid as before. Figure 1 illustrates the effects of distention in one of these vagotomized animals. This figure also illustrates that the time required for the reflex may be less than the interval from the end of one rhythmic contraction until the beginning of the next. This result indicates that the inhibitory effects of distention may be mediated from one loop to the other within as little as $2\frac{1}{2}$ seconds. The vagotomized animals also show awareness of the onset and duration of the distention.

II. *Effect of abdominal sympathectomy on the intestino-intestinal inhibitory reflex.* The method of sympathectomy employed resulted in complete decentralization of the coeliac ganglia and other pre-aortic ganglia, but the connections of each of the Thiry fistulae with these ganglia were intact. The vagi were also intact.

The first three animals were abdominally sympathectomized as follows. A hemostat was clamped on the major splanchnic nerve of the nembutalized dog being given artificial respiration; the nerve was sectioned peripheral to the point of clamping and was followed up under the crus of the diaphragm to its lowermost connection with the sympathetic chain; the chain was cut above this point and was loosened from this point on down into the lumbar region as far as possible and pulled out. After one to two weeks this operation was repeated on the other side. Abdominal sympathectomy by this method resulted in elimination of the intestino-intestinal inhibitory reflex even though the distention were more severe than that used to elicit complete inhibition in the same animal before the operation. This result is illustrated in figure 2. These animals were unaware of the time or degree of the distention.

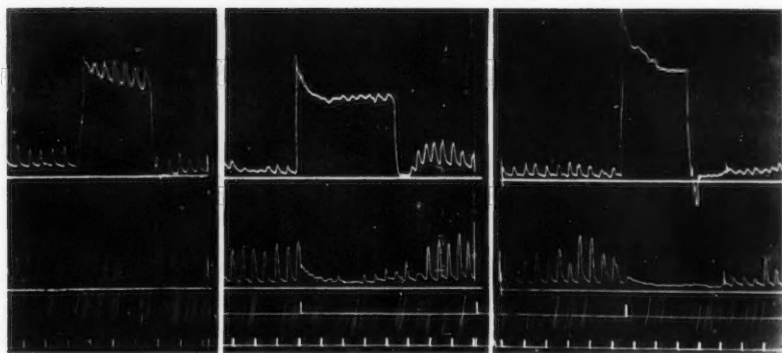


Fig. 1. The intestino-intestinal inhibitory reflex in a vagotomized unanesthetized dog. From above downward there is illustrated 1, balloon-mercury manometer record of motility of a segment of intestine in the form of a Thiry fistula; 2, zero pressure level; 3, motility of a second intestinal segment recorded by a second manometer system; 4, zero pressure level; 5, record of pain response to distention marked by the upstroke, and 6, time in 10 second intervals.

The record on the left shows the effect of introducing 6 cc. of water into the manometer system from which the upper motility record was taken. The sharp decrease in pressure marks the withdrawal of the water. The middle and right-hand records were produced by similar procedures, but 8 and 12 cc. respectively were used.

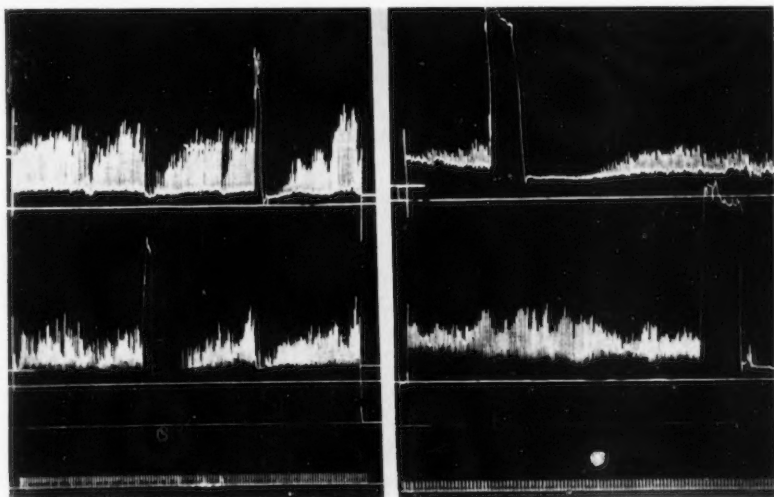


Fig. 2. Elimination of the intestino-intestinal inhibitory reflex by sympathectomy. Vagi are intact. From above downward the writing points are arranged as in figure 1, but the pain responses are not recorded.

The record on the left, obtained prior to the sympathectomy, illustrates that distention of either intestinal segment results in immediate inhibition of the other segment. Right and left sided sympathectomies were done two weeks apart, and the record on the right was made three weeks after the last sympathectomy. Distention of either segment with a pressure of 150 mm. Hg failed to cause inhibition in the other segment.

Kuntz and Van Buskirk (5) have recently reported that reflex inhibition of the intestine is obtained during intestinal distention in nembutalized cats having the coeliac ganglia decentralized. They interpret their experiments as indicating that reflex connections are made through the decentralized coeliac ganglia. The present experiments fail to indicate any mediation of the intestino-intestinal inhibitory reflex through the decentralized pre-aortic ganglia and plexuses even though no anesthetic drugs have been administered. Moreover, Morin and Vial (8) have reported that only splanchnicotomy is necessary to eliminate the intestino-intestinal inhibitory reflex in chloralosed dogs, and Lalieh, Meek and Herrin (6) observed no intestino-gastric inhibitory reflex in unanesthetized dogs when the vagi were cut and the coeliac ganglia and plexuses were decentralized by splanchnicotomy and removal of the lumbar chains. There are experiments which indicate that bilateral splanchnicotomy does not always result in elimination of all of the pathways to the central nervous system from the intestine in vagotomized dogs. Herrin and Meek (3) found that anorexia developed in 3 of 5 dogs which were bilaterally vagotomized and splanchnicotomized and the lumbar chains sectioned; but when the lumbar chains were removed, in addition to the other operations, distention produced no symptoms in any of six dogs. Kuntz and Van Buskirk (5) obtained the intestino-intestinal inhibitory reflex in a part of their animals after splanchnicotomy. It is possible that the reflex inhibition observed in these animals was mediated by ordinary reflexes through the central nervous system by means of connections through the lumbar sympathetic chains.

Two dogs were abdominally sympathectomized by a method which left a possibility of the minor splanchnic nerves remaining intact. The intestino-intestinal inhibitory reflex was almost completely eliminated in one of these animals and was somewhat impaired in the other. The animals still showed awareness of the distention. Each of these two animals was vagotomized without eliminating the pain response or the remaining reflex inhibition. In all of the animals studied it was found that elimination of the pain response was achieved by the same operations that eliminated the inhibitory reflex. Such, of course, would not be the case if the reflex were mediated through the decentralized coeliac plexus. It is apparent from these experiments that complete sympathetic denervation of the intestine can not be assumed if the animal shows a pain response to intestinal distention.

The results of the experiments described indicate that only the sympathetic division of the autonomic system contains both afferent and efferent pathways for the intestino-intestinal inhibitory reflex. The vagus does not contain both limbs of the reflex, but the conditions of the experiment do not test whether it contains one or the other alone. The results reported by Lalieh, Meek and Herrin (6) indicate that the intestinal vagus contains afferent fibers that are activated by intestinal distention, since the intestino-gastric reflex remained after splanchnicotomy and removal of lumbar sympathetic chains. These latter facts suggest that distention of the intestine fails to cause reflex inhibition

of the intestine in the sympathectomized animals because of lack of an efferent inhibitory pathway in the intestinal vagus.

III. *Effect of vagotomy and of sympathectomy on the motor responses of the intestine at the site of distention.* Sudden filling of an intestinal segment having both vagal and sympathetic pathways cut in the mesenteric pedicle evokes a contractile response of the intestine resulting in pressure considerably higher than that produced passively by the introduction of the water (10). Such responses do not occur in the innervated intestine when the pressure produced passively is sufficient to evoke the intestino-intestinal inhibitory reflex. This result has been interpreted as meaning that distention has a direct stimulatory effect on the smooth muscle at the site of distention, but this stimulatory effect is more than counteracted by the long inhibitory reflex. Additional evidence is presented below that the site of distention is under reflex inhibitory influences; and, in accordance with the facts presented in sections I and II, sympathetic denervation alone but not vagotomy alone, unmasks the direct stimulatory effect of distention on intestinal motility.

Mild pressure in the intact dog intestine has a stimulatory effect on intestinal motility (1). The pressure required to elicit the intestino-intestinal inhibitory reflex is relatively severe, ranging from 35 to 100 mm. Hg, even when the sensitivity of the reflex is not depressed by anesthetizing drugs. When the pressure is sufficient to produce reflex amotility of an undistended segment the distended loop also ceases its motility in animals having the sympathetic pathways intact. Figure 1, made from a vagotomized dog, but also typical for animals having all nerve pathways intact, illustrates that introduction of 6 cc. of water into the balloon-manometer system failed to cause inhibition of either the distended or undistended segment. However, after a latent period of several seconds the animal showed a pain response. Introduction of 8 cc. of water, illustrated in the middle record, resulted in partial inhibition of both segments and in an immediate pain response. Introduction of 12 cc. of water resulted in complete inhibition of both segments, and the inhibition gradually died away after removal of the distention. The passively produced pressure falls off rapidly in the distended segment. The failure of the distended segment to contract in response to the stretch stimulus is not attributable to insufficient power on the part of the smooth muscle, since the denervated intestine can actively produce pressures 50 to 100 per cent greater. The immediate inhibition of the distended intestine is attributable to the intestino-intestinal inhibitory reflex, but local inhibitory influences also develop (14) when the distention is maintained. The persistence of inhibition in the undistended segment after removal of the distention of the other segment may be explained possibly by persistence of nervous activity after removal of the distention or by the gradual destruction of the chemical mediator at the neuro-effector junction. The post-distention inhibition in the segment having been distended is the result in part of the same causes as the coinciding inhibition in the non-distended segment, but other local factors are involved since the inhibition is still present in denervated segments (illus-

trated in fig. 2 on the right), and it is commonly more prolonged than the inhibition of the undistended segment. Since the pressures used in the denervated segments are sufficiently great to completely block blood flow through the intestinal wall for the duration of the distention, it is likely that ischemia is a factor in the production of the post-distention inhibition.

The intestine of an abdominally (pre-ganglionically) sympathectomized animal responds locally to distention in the same manner as an intestinal segment that has been completely denervated in the mesentery, while the intestine of a vagotomized animal responds as a normally innervated segment. The two

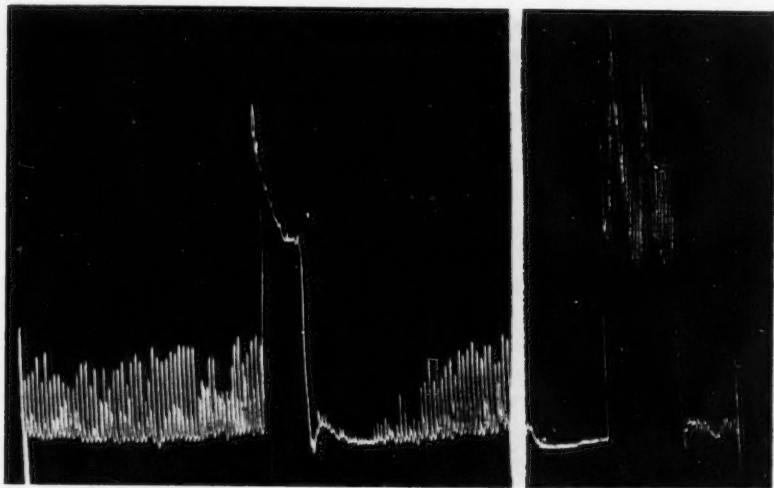


Fig. 3. Elimination of the local intestino-intestinal inhibitory reflex by sympathectomy.

The record on the left illustrates the rapid falling off of pressure and the cessation of contractions that occurs in a normally innervated segment of intestine following the pressure produced by rapid introduction of water into a sidearm of the manometer system. The same result is obtained when only sympathetic pathways are intact (as illustrated in fig. 1, center and right-hand records).

The record on the right shows the stimulatory effects of a similar pressure when the sympathetic pathways to the segment have been preganglionically interrupted. The same result is obtained when all of the nerves in the mesenteric pedicle supplying the segment are cut.

types of response are illustrated in figure 3. These facts are in accord with the following conclusions concerning the intestino-intestinal inhibitory reflex. 1. Only the sympathetic division of the autonomic nervous system contains both afferent and efferent pathways for the reflex. 2. The site of the distention, as well as the intestine for some distance above and below the distention, is involved. 3. The reflex is not mediated through the decentralized plexus either as an ordinary reflex or as an axon reflex.

IV. "*Spontaneous*" development of high pressure in the sympathetically denervated intestine. If the intestine is subjected to a humoral or chemical inhibitory

influence and a force is being exerted from within the intestinal lumen, distention of the intestine will result even though the force be slight. When the inhibitory influence quickly disappears the distended intestine is free to respond to the local stretch stimulus. This response, if it tends to be excessive, is damped by the intestino-intestinal inhibitory reflex when the sympathetic nervous pathways are intact; otherwise the pressure developed may be quite high. Sympathetic denervation, in addition to eliminating reflex control of intestinal pressure, sensitizes the intestine to the humoral products of the sympatho-adrenal system

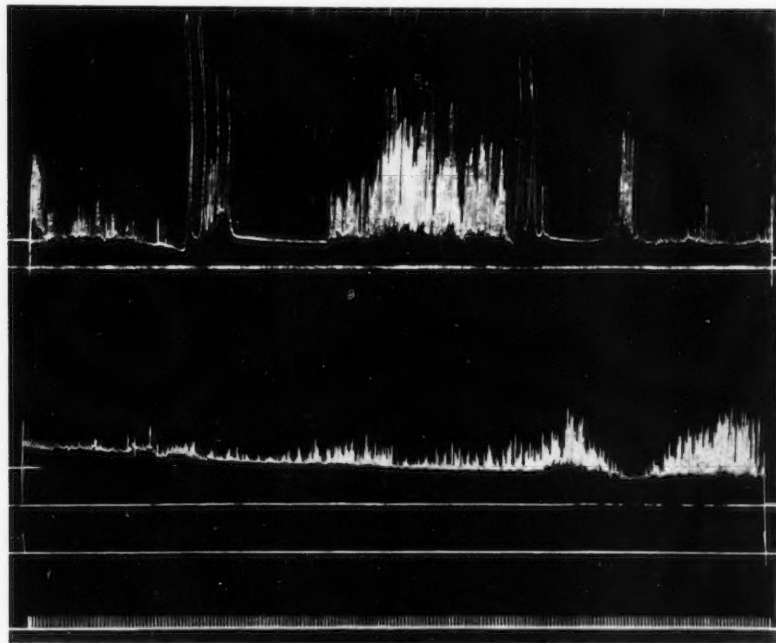


Fig. 4. "Spontaneous" development of pressures of 130 to 150 mm. Hg in the sympathetically denervated intestine.

The upper record is made from a segment having the nerves in the mesenteric pedicle cut, and the lower record is taken simultaneously from a segment having only its sympathetic pathways intact. Time in 10 second intervals. Further discussion in text.

(9). The sensitization is, however, greater after postganglionic sympathectomy than after preganglionic sympathectomy (12). Blood levels of adrenalin and sympathin sufficient to inhibit the sensitized intestine are attained in unanesthetized dogs during psychic disturbances and during widespread reflex activation of adrenergic nerves or reflex activation of the adrenal medulla (11)(12). The denervated intestine becomes distended during the brief time that it is humorally inhibited, and it responds excessively to the distention when the inhibitory influence is quickly removed. Figure 4 illustrates the sudden development of pressures of 130 to 150 mm. Hg following "spontaneous" periods of

inhibition in an extrinsically denervated segment of intestine. Local inhibitory influences apparently develop as a result of the high pressure. None of these irregularities are present in the record made simultaneously from another loop of intestine having only its sympathetic pathways intact. Similar excessive pressures are frequently induced in the denervated intestine following the distention of it which results from intravenous injection of an inhibitory compound. Figure 5 illustrates this latter type of response.

The irregularity of motility of the intestine observed after postganglionic sympathectomy is largely understandable on the basis of sensitization of the

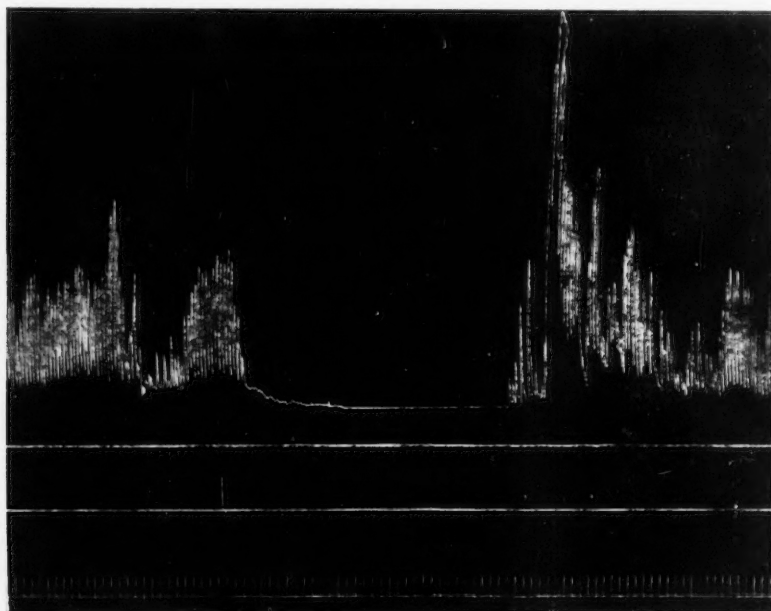


Fig. 5. Development of pressure of 150 mm. Hg in a sympathetically denervated intestinal segment following a period of inhibition produced by intravenous injection of $\frac{2}{3}$ of a unit of pitressin. Vagi are intact. Time in 10 second intervals. Further discussion in text.

intestine and elimination of the intestino-intestinal inhibitory reflex. Once high pressures develop the local inhibitory influences which are associated with excessive pressure in the intestine contribute to the continuance of the irregularity. Ischemia is probably an important factor in the local production of inhibition by high pressures.

SUMMARY AND CONCLUSIONS

The relation of vagal and of sympathetic nervous pathways to the effects of intestinal distention on intestinal motility has been studied by the use of unanesthetized dogs each having two high jejunal fistulae.

The immediate nervous inhibition of one segment of intestine following the sudden production of a pressure of 40 or more mm. Hg in the other segment, referred to as the intestino-intestinal inhibitory reflex, is abolished by sympathectomy alone. The reflex is not noticeably altered by vagotomy. Therefore, only the sympathetic nervous system contains both afferent and efferent pathways for the reflex.

The intestino-intestinal inhibitory reflex is not mediated through the decentralized coeliac ganglia or through other pre-aortic ganglia in unanesthetized dogs even though all the nervous connections between these ganglia and the intestine be intact.

A powerful contractile response may be induced by sudden distention of the sympathetically denervated intestine whether the vagi be intact or not. This type of motor response is not present in the normal intestine because the intestino-intestinal inhibitory reflex involves the site of the distention as well as the undistended intestine above and below the distention.

The irregularity of the motility of the sympathetically denervated intestine is understandable largely on the basis of hypersensitivity to the inhibitory action of adrenalin and sympathin, elimination of the intestino-intestinal inhibitory reflex, and the development of local inhibitory influences as a result of high intra-luminal pressure.

The regulation of intestinal motility by its extrinsic nerves consists, in part, of reflex inhibition of the intestine as a result of stimuli arising from excessively strong intestinal contractions. One function of this reflex is that it helps to keep the pressure within the intestine below the level which blocks blood flow through the vessels of the intestinal wall.

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CEPHALOGYRIC REACTIONS OF NON-LABYRINTHINE ORIGIN

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The experiments reported here were instigated by observations made on decerebrate cats in which the effects of galvanic stimulation of the labyrinth were studied; 41 cats were used in this investigation. In decerebrate cats bilateral section of the eighth nerves reversed the direction of the rotation of the head about the oro-occipital axis produced by direct current stimulus with the anode in the one and the cathode in the other external auditory meatus. Before the section of the eighth nerves this rotation was directed to the anode, as is regarded typical for galvanic stimulation of the labyrinth, while it was directed to the cathode after these nerves had been severed (threshold on good preparations 3-6 milliamperes). This rotation to the cathode was sometimes associated with a turning of the head about the dorsoventral axis to the opposite shoulder (particularly if strong currents were employed—up to 10 milliamperes). A similar reversal of direction of rotation following the section of the eighth nerve was observed when the stimulation (with direct current, rectangular alternating current, or faradic current) was performed by a bipolar electrode of the concentric needle type placed into the round window on the promontory, the rotation being directed to the opposite side before, and to the same side after, section of the corresponding eighth nerve. Again the rotation to the same side sometimes was combined with turning of the head to the opposite shoulder. One may, perhaps, briefly call the rotation to the anode on stimulation with a current flowing transversely through the skull, and the rotation to the opposite side with a bipolar electrode in the round window "contralateral cephalogyric reaction" and consider it as due to stimulation of the peripheral neuron of the vestibular nerve (Dohlman, 1929), while the rotation to the cathode on transverse stimulation of the head or to the side of stimulation on unilateral bipolar stimulation may be called "ipsilateral cephalogyric reaction". Since this ipsilateral rotation appears after the eighth nerve has been cut and persists also after bilateral lesion of the vestibular nuclei (see later) it may be regarded as non-labyrinthine in origin.

In some instances the ipsilateral rotation was observed with the eighth nerves still intact, for instance, if the animal lost much blood during the decerebration and the excitability of the labyrinth was decreased, or if the galvanic stimulus was repeatedly applied (exhaustion of the labyrinthine reaction). Occasionally the rotation to the anode appeared on stimulation with weak currents (1-2 milliamperes) and the opposite reaction with strong currents (4 milliamperes and

above, on bipolar stimulation). On bipolar stimulation with a rectangular alternating current the reversal could be obtained in single cases by reducing the duration of the single phases, e.g., in cat XX rotation to the opposite side appeared with stimuli above 2.7 msec. and to the same side below 0.9 msec. Such a result was, however, not regularly obtained, most animals with the eighth nerve intact reacting only with rotation to the opposite side, until a liminal duration (0.9–1.8 msec.) was reached below which no reaction appeared at all.

After unilateral severance of the eighth nerve on stimulation with a D.C. flowing transversely through the skull the most frequent reaction was rotation to the anode, if the cathode lay on the side of the intact nerve, and a rotation to the cathode, if the latter was on the side of the cut nerve. In one case (XIV) a slight rotation to the anode or at least a diminution of the spontaneous rotation of the head to the side of the cut eighth nerve could be observed also when the cathode was on the side of the nerve section. A similar observation was transitorily made in a second case (XXII), which showed, however, on repeated examination the first mentioned type of reaction. Occasionally stimulation with the cathode on the side of the cut eighth nerve failed to produce a definite effect as long as the current was on, while a rotation to the anode appeared on interruption of the current (cat XXX).

In an effort to analyse the mechanism of the ipsilateral rotation, the following data could be obtained. The reaction was still present after extirpation of the mid-brain with the red nucleus, after ablation of the cerebellum, after bilateral lesion of the vestibular nuclei by incision on the inner aspect of the corpus restiforme. Extirpation of medulla oblongata and pons in animals kept under artificial respiration abolished it. Also it could no longer be obtained in decerebrate cats in whom, in addition to both eighth nerves, the roots of the fifth and of the ninth to eleventh were cut bilaterally. The only effects of stimulation with strong currents (15 milliamperes) observed in such preparations on the neck were bilateral contractions of the muscles in the back of the neck which failed, however, to produce definite rotation and resulted only in retraction of the head. One may infer that the ipsilateral rotation is not due to a direct stimulation of neck muscles or of peripheral nerves and is also not caused by reflexes for which the spinal cord is sufficient, but that one deals with a stimulation of the oblongata and pons. This may be a direct stimulation of cell groups of the rhombencephalon by the electric current or a reflex stimulation.

A direct stimulating action of the current upon the cell groups of the oblongata or pons can be excluded for the following reason. It will be shown below that the efferent pathway of the ipsilateral rotation takes its way, at least partly, over fibers descending to the spinal cord. After the roots of the fifth to eleventh nerves had been bilaterally cut, electric stimulation failed to elicit the ipsilateral rotation although the efferent pathways to the spinal cord were still present. This seems to indicate that one deals with a reflex mechanism, elicited by afferent impulses that enter oblongata and pons. This view is also corroborated by the following experiences. In decorticated cats with both 8th nerves cut a transversely flowing current of 2.5 milliamperes was able to produce ipsilateral

rotation while increase of the current up to 15 milliamperes failed to produce eye movements. The motor nerves of the eyeballs were intact in these preparations, as was shown by the good contraction of the sphincter of the pupil and its reactivity to reflex stimuli. If the current had stimulated the rhombencephalon, such stimulation should have reached the vestibular nuclei and their connections with the eye muscles so that ocular movements had appeared.

In order to ascertain which afferent fibers are responsible for this reaction, two groups of experiments were performed. In the first the 8th and 9th-11th roots were cut on both sides; the ipsilateral rotation could still be elicited, indicating that afferent impulses conducted by trigeminal fibers play a part in the mediation of this reaction. In the second series of experiments the severance of both eighth nerves was combined with that of both fifth roots. Also these operations did not prevent the appearance of the ipsilateral cephalogyric reaction.¹ The rotation of the head could be ascribed in these latter experiments either to a reflex stimulation of afferent fibers of the 9th-10th nerves,¹ or to a direct stimulation of efferent fibers of the 11th nerves. Since cutting all roots from the fifth to eleventh abolished the reaction, and it had, therefore, to be considered as a reflex, the ipsilateral rotation appearing on electric stimulation after section of the 5th and 8th roots is due to reflex stimulation of afferent 9th-10th fibers. Thus, stimulation of afferent 5th as well as of afferent 9th-10th fibers participates in the ipsilateral cephalogyric reaction. If electrodes are placed in the external ears so that an electrical current flows transversely through the skull, such a current could stimulate nerve endings in the external or middle ear or the roots of the 5th, 9th and 10th nerves within the intracranial cavity. Similar possibilities come into question if bipolar electrodes are placed on the round window. The effect of bipolar stimulation of the external meatus and of the middle ear was therefore studied. Faradic stimulation of the external auditory meatus in decerebrate cats produced retraction of the head and occasionally rotatory reactions of irregular direction, but these reactions appeared only if strong currents were employed (coil distance 6 cm.) and had, therefore, to be ascribed to an escape of the current. Stimulation of the mucous membrane of the bulla ossea and of the tympanic cavity also failed to produce a rotation in decerebrate cats except if the electrodes were placed close to the adjacent accessory nerve so that the nerve was stimulated by an escape of the current; this latter effect was abolished by cutting the 11th nerve.

Thus, the ipsilateral cephalogyric reaction appearing on electrical stimulation as described is not due to stimulation of branches of the 5th or 9th and 10th nerves in the external or middle ear, but the current apparently stimulates the afferent fibers more centrally, probably within the roots of these nerves. Such an interpretation is corroborated by experiences on the mechanism of the contralateral (vestibular) cephalogyric reaction. This latter reaction persists after destruction of the labyrinth, but is abolished by severance of the 8th nerves; it

¹ The term 9th-10th nerves is here used for the sake of brevity, instead of 9th and/or 10th nerves; a differentiation of the afferent fibers of these two nerves by separately cutting the respective roots was not attempted.

should therefore be ascribed to a stimulation of the 8th nerve or its roots respectively (Spiegel). If one correlates these experiences on the ipsilateral and on the contralateral cephalogyric reaction, one may arrive at the following conception. If a current flows transversely through the skull between the ears, or if it is applied by introducing a bipolar electrode into the inner ear, the current is able to stimulate not only the 8th nerve but also the adjacent roots of the 5th as well as the 9th-10th nerves. As long as the 8th nerve is excitable the vestibular reaction (contralateral rotation) prevails, while the effect upon the neighboring sensory roots producing reflexly the ipsilateral rotation becomes apparent when the 8th nerve is cut or has lost its excitability.

As for the efferent pathways, the fact that after bilateral severance of the 8th-11th roots an ipsilateral rotation could still be elicited indicates that afferent trigeminal impulses may induce the reaction by way of fibers descending from the rhombencephalon to the spinal cord. A similar efferent mechanism may also be used by the impulses entering the brain stem with the 9th and 10th roots. This is indicated by the following experience. With the 5th and 8th nerves bilaterally cut, the ipsilateral rotation may still be elicited after the accessory nerves have been severed in the neck; additional transverse section of the uppermost cervical segment abolishes the reaction. The accessory nerve, however, also carries efferent impulses producing the ipsilateral rotation that is elicited by stimulation of afferent 9th-10th fibers, since the reaction can still be observed with the 5th and 8th nerves bilaterally cut and the connection between oblongata and spinal cord severed; subsequent section of the accessory nerve in the neck abolishes the rotation to the corresponding side. In order to ascertain whether the ipsilateral rotation elicited by stimulation of afferent trigeminal fibers may be produced by way of efferent impulses along the accessory nerve, the 8th nerves were bilaterally cut, then the root of one fifth nerve was stimulated (faradic current, bipolar electrode), before and after high transverse section of the cervical cord. In some experiments the ipsilateral rotation could no longer be elicited after section of the cord, in some cases it was present, but only if strong currents were employed (coil distance 6 cm.) so that an escape of the current to the roots of the accessory nerve could not be precluded. Thus, for the ipsilateral rotation produced by stimulation of afferent trigeminal fibers an efferent pathway using the accessory nerve could not definitely be established.

The knowledge of these cephalogyric reactions may, perhaps, contribute to a better understanding of the mechanism of some types of torticollis.

SUMMARY

1. Bilateral section of the eighth nerves in decerebrate cats reverses the direction of the rotation of the head produced by a D.C. that flows transversely through the skull; the rotation being directed toward the anode before, and toward the cathode after section of the 8th nerves.

2. After unilateral section of the 8th nerve in decerebrate cats usually the rotation to the anode is preserved when the cathode lies on the normal side, while the rotation to the cathode may appear, if the latter lies on the operated

side. On bipolar monaural stimulation one observes rotation to the opposite side as long as the 8th nerves are intact, and to the same side after the corresponding nerve has been cut.

3. The rotation to the anode on transverse binaural stimulation or to the opposite side on bipolar monaural stimulation (contralateral cephalogyric reaction) is a vestibular reaction. The rotation to the cathode and to the same side respectively (ipsilateral cephalogyric reaction) is due to stimulation of afferent fibers in the roots of the 5th and of the 9th-10th nerves respectively; it may appear not only if the vestibular nerves are cut but also if their excitability is depressed.

4. The centers of the ipsilateral cephalogyric reaction lie in oblongata and pons. The reaction elicited by stimulation of afferent trigeminal fibers uses efferent fibers descending into the spinal cord; an efferent pathway along the accessory could not be established with certainty for this part of the reaction. For the ipsilateral rotation elicited by stimulation of afferent 9th-10th fibers efferent pathways could be demonstrated using the accessory nerve as well as fibers descending into the spinal cord.

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SOME EFFECTS OF PROSTIGMINE AND ACETYLCHOLINE ON CORTICAL POTENTIALS

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Early investigators of the pharmacology of physostigmine (cf. Langley and Kato, 1915) noticed its grossly apparent central excitatory effects. Later investigators described changes in reflex activity after injection of eserine and acetylcholine (Dikshit, 1934; Schweitzer and Wright, 1937). Changes in the electrical excitability of the motor cortex (Miller, 1937; McKail, Obrador and Wilson, 1941) and in the electrocorticogram (Sjöstrand, 1937; Bonnet and Bremer, 1937a, b; and Miller, Stavrakys and Woonton, 1940) have been produced by local application or injection of the drugs.

The present study is a further investigation of the changes produced in the electrocorticogram of cats by prostigmine and acetylcholine, with special emphasis on those recorded from somesthetic sensory areas.

METHODS. Cats, anesthetized with nembutal (0.7 cc. per kgm.), were used. Bipolar silver wire electrodes with an interpolar distance of 1 mm. were placed upon the various cortical areas, and recording was accomplished either by a Grass ink-writer or a DuBois oscillograph.

The drugs used were a 1 per cent solution of prostigmine (Roche); 1 per cent acetylcholine chloride (Merek); 1 per cent strychnine sulfate; and atropine sulfate (Sharp and Dohme). The first three substances were applied locally to the cortex by means of small squares of filter paper saturated with the solution and placed on the cortex under the electrodes. Strychnine was removed when its effects had become apparent, while prostigmine and acetylcholine were applied for ten to twenty minutes each. Atropine in solution was injected intravenously.

RESULTS. When acetylcholine alone was applied to the cortex, no noticeable change in the action potentials was observed.

After application of prostigmine, a series of changes in the cortical potentials occurred which may be described as follows. There was at first a depression of the spontaneous activity. This depression sometimes remained localized in the area to which the drug had been applied, but sometimes spread to other cortical areas, reducing the spontaneous activity at points remote from the application (fig. 1 a and b). Later, spontaneous bursts usually reappeared in the records.

Application of acetylcholine to an area previously treated with prostigmine caused further changes in the electrocorticogram. At first, the spontaneous bursts were augmented in size, increasing both in voltage and duration. Concomitantly, the individual potentials within the burst became larger and sharper

in outline, resulting in the appearance of spikes rather than waves. Other spikes appeared in the intervals between the bursts until the electrocorticogram consisted of a continuous series of spikes at a frequency of 5-10 per second (fig. 1 c). Still later, fast (20-30 per sec.) lower voltage potentials also appeared in the record (fig. 1 e and d).

A. *Cortical areas in which spikes were induced.* Treatment with prostigmine and acetylcholine was followed by the series of changes described above, when the drugs were applied to the ectosylvian, posterior sigmoid, and anterior sigmoid

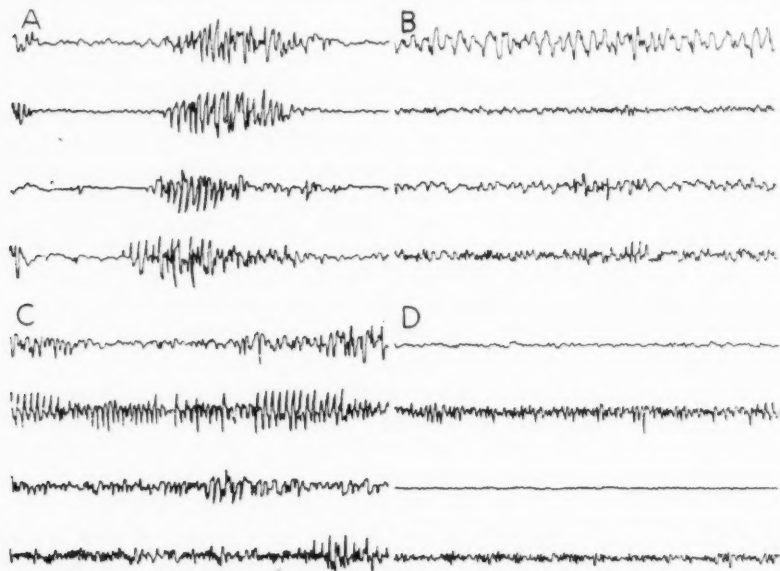


Fig. 1. Effects of prostigmine and acetylcholine on the electrocorticogram.

Records from above downward: 1, sciatic projection. 2, radial projection (R_2 of Marshall, 1941). 3, neighboring radial projection (R_3 of Marshall). 4, anterior suprasylvian gyrus. Bipolar recording. Paper speed 7.5 mm. per sec.

A, normal activity. B, after local application of prostigmine under electrode 2 (R_2 of Marshall). C, after subsequent application of acetylcholine. Note 5-10 per sec. spikes and fast activity at point of application, and fast activity in suprasylvian gyrus (bottom record). D, after section of thalamic radiations. The 5-10 per sec. spikes have been abolished, but fast activity remains in both the treated area and the suprasylvian gyrus.

gyri. The electrode placement on these gyri was such that potentials were recorded on the ectosylvian after auditory stimulation, on the posterior sigmoid after radial or sciatic nerve stimulation, and on the anterior sigmoid after brachium conjunctivum stimulation (cf. Morison and Dempsey, 1941). Although it is well known that the anatomical limits of the gyri do not correspond to their functional divisions, it is convenient throughout the remainder of this paper to refer to the ectosylvian gyrus as "acoustic" cortex, to the posterior sigmoid as "somesthetic" cortex, and to the anterior sigmoid as "motor" cortex.

Application of the drugs to the anterior, middle and posterior marginal, or the middle suprasylvian gyri never produced either the 5-10 per second spikes or the rapid low voltage activity. Treatment of these latter areas, however, frequently increased the size of the potentials in the spontaneous bursts.

B. Spread of activity in the cortex. Local application of prostigmine and acetylcholine to the somesthetic, motor, or auditory cortex, with simultaneous recording from other cortical areas, revealed that the 5-10 per second spikes were sharply localized to the area treated. The later development of fast low voltage waves spread from the treated area to corresponding motor and association areas of the same hemisphere, but not to adjacent untreated somesthetic areas nor to any regions of the contralateral cortex (figs. 1c, 1d and 2b).

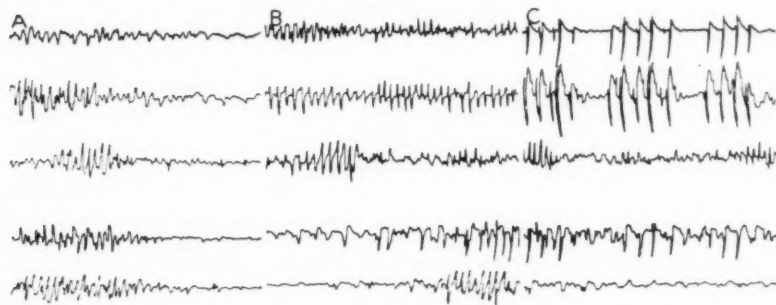


Fig. 2. Comparison of spread of activity after prostigmine-acetylcholine and strychnine. Records from above downward: 1, left anterior sigmoid gyrus. 2, left sciatic projection. 3, left radial projection (R_2 of Marshall). 4, right sciatic projection. 5, right radial projection (R_2 of Marshall). Bipolar recording. Paper speed 7.5 mm. per sec.

A, normal activity. B, after application of prostigmine and acetylcholine to left sciatic projection. Note presence of fast activity in the homolateral anterior sigmoid gyrus. C, after application of strychnine to left sciatic projection. Note presence of "strychnine spikes" in homolateral anterior sigmoid gyrus and in contralateral sciatic projection (record 4).

C. Effects upon production of sensory cortical potentials. Stimulation of peripheral nerves causes the appearance of cortical potentials which are sharply localized in the region of the sensory cortex corresponding to the area stimulated (Marshall, Woolsey and Bard, 1937; Forbes and Morison, 1939). After local treatment of the somesthetic cortex with prostigmine and acetylcholine, these "primary" responses to peripheral nerve stimulation could still be induced (fig. 3). The potentials resulting from single shocks were either unaffected or slightly reduced in size.

Repetitive stimulation of a nerve revealed that the primary response declined somewhat faster than normal as the frequency of stimulation was increased. Moreover, on repetitive stimulation a response appeared whose latency was longer than that of the normal primary (fig. 3d). This long-latency primary had rather slow frequency characteristics, showing almost complete alternation at stimulation frequencies of about 7 per second.

In contrast to the sharp localization of sensory potentials in normal animals, stimulation of peripheral nerves after treatment of the corresponding area of the somesthetic cortex with prostigmine and acetylcholine led to the appearance of potentials in other areas of the cortex as well. Figure 4a illustrates a potential recorded from the motor cortex after treatment of the somesthetic cortex and stimulation of the corresponding nerve. However, such treatment apparently does not open up paths to all the cortical areas which are known to receive fibers from the somesthetic area. Figures 2c and 4 show that while strychninization of the somesthetic cortex leads to the appearance of potentials in both the homolateral motor and contralateral somesthetic areas, similar application of prostigmine and acetylcholine does not alter the activity of the contralateral somesthetic area.

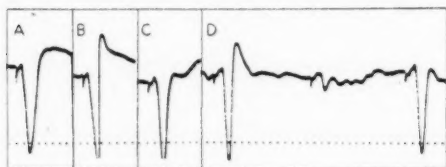


Fig. 3

Fig. 3. Effects of prostigmine-acetylcholine on primary response to peripheral stimulation. Records from radial projection area (R_2 of Marshall), stimulus to contralateral radial nerve. Time intervals, 10 msec.

A, normal response. B, response after application of prostigmine. C, response to single shock after subsequent application of acetylcholine. D, responses to repetitive stimulation (about 7 per sec.). Note increased latency and alternation of response.

Fig. 4. Comparison of prostigmine-acetylcholine and strychnine effects on spread of response to peripheral stimulation. Stimulus to right sciatic nerve, drugs were applied to the left sciatic projection. Time intervals, 10 msec.

A, record from left anterior sigmoid gyrus after application of prostigmine-acetylcholine to homolateral radial projection. B, record from left anterior sigmoid gyrus after application of strychnine to homolateral radial projection. C, record from right sciatic projection after application of prostigmine-acetylcholine to left sciatic projection. D, record from right sciatic projection after application of strychnine to left sciatic projection.

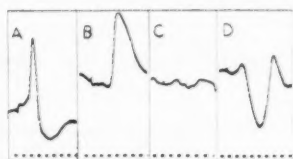


Fig. 4

Under light nembutal anesthesia, the primary sensory potential is sometimes followed by a train or burst of repetitive responses which, like the primary potential itself, is also sharply localized in the sensory cortex (Morison and Dempsey, unpublished data). This effect became quite striking when prostigmine and acetylcholine were applied to the somesthetic cortex. After such treatment, the magnitude and duration of this repetitive train of responses were markedly increased (fig. 5).

D. *Effects of atropine.* Atropine (1 mgm. per kgm., I.V.) abolished the 5-10 per second spikes occurring in the intervals between bursts, but did not affect the spike components of the spontaneous bursts (fig. 6).

The rapid low voltage activity induced in treated and remote regions was not abolished by atropine, nor was the spontaneous activity of the untreated cortex affected. The response to peripheral nerve stimulation elicitable from the motor

region after treatment of the corresponding somesthetic cortex with prostigmine and acetylcholine likewise was unaffected by atropinization of the animal.

E. *Effect of section of thalamic radiations.* Isolation of the treated cortex either by undercutting or by removal of the thalamus abolished all components of the prostigmine-acetylcholine effects except the fast low voltage waves. The latter not only remained in the treated area, but in the motor and association cortices as well (fig. 1d).

DISCUSSION. It has recently been shown that the spontaneous ECG of cats under nembutal anesthesia may be divided, on the basis of physiological criteria, into component parts which can be analyzed separately (Morison and Dempsey, 1941). The results cited in the preceding sections lend further support to this point of view, since it was shown that various elements of the activity resulting from treatment with prostigmine and acetylcholine are differently affected by



Fig. 5. Increased repetitive response to peripheral stimulation after prostigmine and acetylcholine.

Records from left radial projection, single stimuli signaled by ↓ to right radial nerve. Paper speed, 10 mm. per sec.

A, normal. B, after application of prostigmine. C, after subsequent application of acetylcholine.

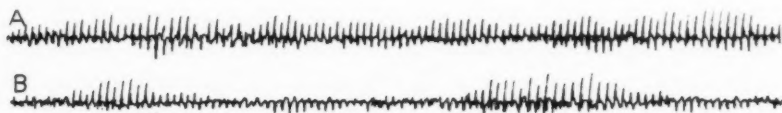


Fig. 6. Effects of atropine on prostigmine-acetylcholine potentials. Records from sciatic projection. Paper speed, 7.5 mm. per sec.

A, activity after application of prostigmine and acetylcholine. B, after intravenous injection of atropine (1 mgm. per kgm.). Note disappearance of "interval" spikes.

different experimental procedures. For example, section of the thalamic radiations abolishes all prostigmine-acetylcholine effects except the rapid low voltage waves (fig. 1d). The latter effect represents, therefore, activity which is intrinsic in the cortex and does not require the participation of thalamic circuits. On the other hand, the activity associated with spontaneous bursts and the continuous 5-10 per second spikes are abolished by removal of the thalamus (fig. 1c and d). Presumably, then, these effects represent activity in thalamo-cortical circuits.

A separation of the total activity into component parts can also be made on the basis of the effect of atropine. Atropine abolishes the 5-10 per second "interval" spikes but does not affect the augmented bursts (fig. 6), the rapid low voltage activity, nor the primary response recordable from the motor cortex (p. 636). From the standpoint of classical pharmacology, this indicates that

both the muscarine and nicotine effects of acetylcholine may be observed in the central nervous system.

Acetylcholine apparently opens up pathways in the cortex which formerly did not respond. This characteristic is demonstrated in figure 4, which shows a potential recorded from the motor cortex after treatment of the somesthetic cortex and stimulation of a peripheral nerve. The observation that a relatively long latency response appears in the somesthetic cortex during such experiments, even though the true primary response appears to be more labile than normal (fig. 3), reinforces this conclusion. Such spread of activity is comparable to the well-known "lowering of synaptic resistance" induced by strychnine. The acetylcholine effect is more selective than is that of strychnine, however, since the latter drug also opens up paths to the contralateral cortex while acetylcholine does not (figs. 2 and 4).

The production by prostigmine and acetylcholine of characteristic changes in the cortical potentials raises questions of the levels at which their effects occur. Since the effects are brought about by local cortical treatment, the cortex is obviously suspect as the locale in question. Furthermore, persistence of the rapid, low voltage potentials after section of the thalamic radiations (fig. 1) conclusively implicates purely cortical elements insofar as this component of the electrical activity is concerned. On the other hand, the 5-10 per sec. activity probably represents activity in a thalamo-cortical mechanism, since it is abolished by section of the thalamic radiations.

The 5-10 per second activity appears grossly similar to either the normal spontaneous bursts of activity or to the repetitive response induced by nerve stimulation in lightly anesthetized preparations (Morison and Dempsey, unpublished data). A tentative identification of the 5-10 per second activity with the repetitive response can be made on the basis of the following similarities in behavior. Their frequencies are the same, and both are sharply localized to the somesthetic cortex. Likewise, figure 5 demonstrates that the acetylcholine effect grows out of the repetitive response as more and more time elapses during treatment. Contrariwise, it is unlikely that the acetylcholine effect is related to the normal spontaneous bursts, since the former can be produced only in sensory and motor areas, while the latter are best developed in the association areas in which acetylcholine spikes cannot be produced (p. 634), and normally occur throughout the cortex.

The sensory and motor areas of the cortex receive specific projection fibers from the thalamic relay nuclei, while the thalamic connections of the association areas probably are more diffuse (Cf. Morison and Dempsey, 1941). Furthermore, it is well known that the sensory and motor areas both send and receive fibers to and from their thalamic nuclei. Finally, it has been found that the repetitive response to sensory stimulation interacts with the primary sensory response (Morison and Dempsey, unpublished data). All these considerations render it likely that the 5-10 per second acetylcholine effect represents increased activity in the specific thalamo-cortical feed-back circuits (cf. Dusser de Barenne and McCulloch, 1938) rather than in the more generalized circuits responsible

for the normal spontaneous bursts of potentials (cf. Morison and Dempsey, 1941).

The present experiments demonstrate that prostigmine and acetylcholine produce changes in the central nervous system which are at least qualitatively similar to the effects which previously have been described in autonomic and neuromuscular systems. For example, the opening up of synaptic paths from the sensory to the motor cortex (fig. 4) is at least roughly comparable to the increased responsiveness of the cervical sympathetic synapse when acetylcholine is administered together with afferent stimulation. Similar effects have been noted in fatigued neuro-muscular systems (Rosenblueth and Morison, 1937). The phenomena reported above indicate that the central nervous system can be studied by analytical procedures similar to those used in simpler systems, and that data can be obtained comparable to those already used in analyzing autonomic and neuromuscular transmission.

SUMMARY

Acetylcholine applied locally to the cerebral cortex of cats produced no change in the electrocorticogram. Prostigmine, similarly applied, was followed by a transient depression of spontaneous activity both at the area of application and elsewhere (fig. 1). A characteristic series of changes in the electrical activity occurred when prostigmine followed by acetylcholine was applied to the somesthetic, auditory and motor cortex, but not when association cortex was similarly treated (p. 635). These changes consisted of increased spontaneous activity, of the appearance of characteristic 5-10 per second spikes, and the later development of rapid (20-30 per sec.) lower voltage potentials. The 5-10 per second spikes remained well localized to the treated cortex, while the rapid activity spread to certain other cortical areas (figs. 1 and 2).

The "primary" response to single stimulation of a peripheral nerve was either unchanged or slightly reduced after treatment (fig. 3), but the repetitive response to single stimulation was greatly increased (fig. 5). On repetitive stimulation of a peripheral nerve, the primary response declined rapidly in magnitude and was followed by a second, longer latency response which alternated at certain stimulus frequencies (fig. 3). After treatment of the somesthetic cortex, a response could be recorded from the corresponding homolateral motor cortex after stimulation of a sensory nerve (fig. 4).

Atropine abolished the spikes occurring in the intervals between bursts, but was without effect on the other changes produced by prostigmine and acetylcholine (fig. 6). Section of the thalamic radiations abolished all the effects of treatment except the rapid low voltage activity (fig. 1).

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FUNDAMENTAL DIFFERENCES IN THE EXCITABILITY OF SOMATIC AND AUTONOMIC CENTERS IN RESPONSE TO ANOXIA

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The fact that whereas cortical processes (Gellhorn and collaborators), sub-cortical (Gellhorn and Storm), and even spinal reflexes (King and collaborators) are depressed in anoxia, the blood pressure rises and the respiratory volume increases, indicates an important difference between somatic and vegetative centers. Since anoxia depresses the respiration as well as the vasomotor center after denervation of the carotid sinuses and bilateral vagotomy (Heymans; Gellhorn and Lambert; Schmidt and Comroe, and others) the difference is due to the influence of the chemoreceptors on the centers. The excitability of autonomic and somatic structures in the hypothalamus, the medulla, and the spinal cord and the rôle of the afferent impulses originating in the chemoreceptors have been studied under the influence of anoxia in order to determine whether fundamental differences exist in the reactivity of autonomic and somatic centers. The experiments have indeed demonstrated the existence of these differences, which are in part inherent in these centers and therefore independent of afferent impulses.

METHODS. The experiments were performed on more than 60 cats narcotized either with chloralose (85 mgm/kgm. subcutaneously) or with 35 mgm/kgm. pentothal² intraperitoneally followed by 35 mgm/kgm. chloralose intravenously. The latter combination was found useful inasmuch as it eliminated the use of ether and maintained a high blood pressure and a good excitability of the nervous system.

The hypothalamus and the vasomotor center in the medulla were stimulated either by a Harvard inductorium or by the secondary of a General Electric Variac whose primary was connected with a 110 V. 60 c.p.s. line. The Horsley-Clarke apparatus was used for the proper placement of the electrodes (cf. Carlson, Gellhorn and Darrow). The spinal cord was exposed at a thoracic level and stimulated with either monopolar (Sherrington) or bipolar platinum electrodes. The oxygen-nitrogen gas mixtures were inhaled from Douglas bags (cf. Gellhorn and Packer). Artificial respiration was employed.

¹ Aided by a grant from the John and Mary R. Markle Foundation. Assistance was also given by the W. P. A. Project, Ill. no. 30278.

² Kindly supplied by the Abbott Laboratories, North Chicago, Illinois.

RESULTS. I. *The effect of anoxia on the excitability of the hypothalamus and the rôle of the buffer nerves.* By means of the Horsley-Clarke apparatus the hypothalamus was explored until a point was found which produced on stimulation a distinct contraction of the nictitating membrane (n.m.). The cervical sympathetic was cut on the contralateral side and the cephalic end was stimulated with an inductorium. The contractions of both n.m. were recorded. It was invariably found that anoxia produced by the inhalation of 4.5 per cent oxygen never altered the contraction of the n.m. which resulted from stimulation of the cervical sympathetic nerve, while at the same time anoxia had a profound influence on the contraction of the n.m. elicited by hypothalamic stimulation.

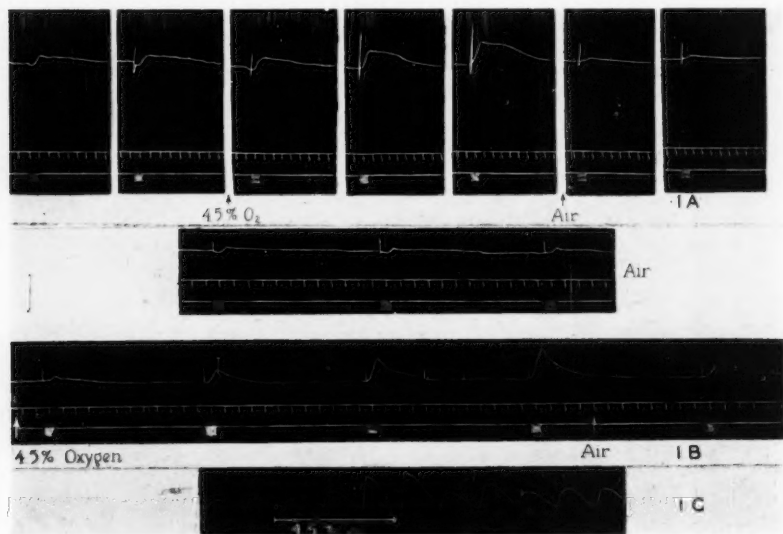


Fig. 1a and 1b. Stimulation of the hypothalamus in intervals of one minute. Harvard inductorium; coil distance 5 cm. Recording of the contraction of the nictitating membrane before, during and after inhalation of 4.5 per cent oxygen.

Fig. 1c. Stimulation of the hypothalamus after denervation of the carotid sinuses and bilateral vagotomy. Harvard inductorium. Coil distance 5.5 cm.

This indicates that the change produced was due to an alteration in excitability of the hypothalamus and not to a change in the reactivity of the peripheral nerve, neuromuscular junction, or the n.m. itself.

Figure 1 shows that inhalation of 4.5 per cent oxygen causes an increase in the contraction of the n.m. on hypothalamic stimulation. The effect is quickly reversible on readmission of air. In the case of figure 1-A it is seen that this increase in central excitability due to anoxia is followed by a post-anoxic depression during which the height of the contraction of the n.m. is smaller than during the control period preceding the experiment. In some other cases, however, (figure 1-B) the post-anoxic depression was absent. Since the n.m. of the cat is in-

nervated by sympathetic fibers only, the experiments indicate that sympathetic hypothalamic centers show an increased excitability during anoxia.

The experiments were repeated after the carotid sinuses³ had been denervated and the vagi cut. Figure 1-C shows that after elimination of the buffer nerves anoxia still produces a greatly increased contraction of the n.m. on hypothalamic stimulation. From numerous experiments of this kind it may be concluded that the sympathetic hypothalamic centers governing the contraction of the n.m. show an increased excitability in anoxia which persists after the elimination of afferent impulses from the chemoreceptors.

The influence of anoxia on the blood pressure response to hypothalamic stimulation was also studied. Figure 2-A shows an experiment in which by means of a weak alternating current (1.2 V) the hypothalamus was stimulated at intervals of two minutes and the blood pressure reaction was recorded through a mercury

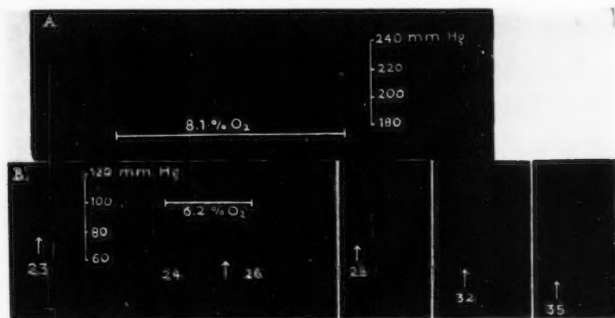


Fig. 2a. Blood pressure from the carotid artery (upper graph). Stimulation of the hypothalamus with the 60 cycle current (5 sec.) at 1.2 volts at intervals of 2 minutes.

Fig. 2b. Blood pressure from the carotid artery (lower graph). Stimulation of the hypothalamus (indicated by arrow) in a cat with carotid sinuses denervated and bilateral vagotomy. Harvard inductorium, coil distance 4.5 cm., duration 10 seconds. At 23 and 28 cat inhaled air, between 24 and 26 it inhaled 6.2 per cent O₂. Prior to 32 and 35 the blood pressure was lowered through bleeding from the femoral artery.

manometer from the carotid artery. It is seen that the pressor response greatly increased during inhalation of 8.1 per cent oxygen. On readmission of air the original pressor response was restored. These results are obtained with regularity provided that no oxygen concentrations are chosen which produce a very great rise in blood pressure per second. Under the latter condition an actual decrease and even an absence of response may be observed.

These experiments were repeated after the carotid sinuses and vagi had been eliminated. In figure 2-B a typical example is reproduced. As was shown by Gellhorn and Lambert, the inhalation of gases low in oxygen produces a fall in blood pressure in proportion to the duration and the severity of anoxia in such animals, whereas in an animal whose chemoreceptors are intact, anoxia regularly

³ This term has been used in this paper to denote the denervation of pressor- and chemoreceptors of the carotid sinus area.

produces an increase in blood pressure. If during the period of anoxia the hypothalamus is stimulated in a "denervated"⁴ animal, it is found that the blood pressure response is now very greatly reduced. This effect is reversible as shown by the fact that on readmission of air the same blood pressure response is obtained on hypothalamic stimulation as was observed before the oxygen-nitrogen mixture was administered.

It is of importance to decide whether or not the decreased reactivity during anoxia is due to the diminution of the blood pressure level or to the absence of impulses from the chemoreceptors. Figure 2-B shows that when the blood pressure is gradually lowered by slight bleeding the reactivity of the vasomotor center is not decreased to a degree comparable to that found on inhalation of gas mixtures low in oxygen in spite of the fact that the fall in blood pressure produced by the bleeding is greater than that obtained under conditions of inhalation of 6.2 per cent oxygen.

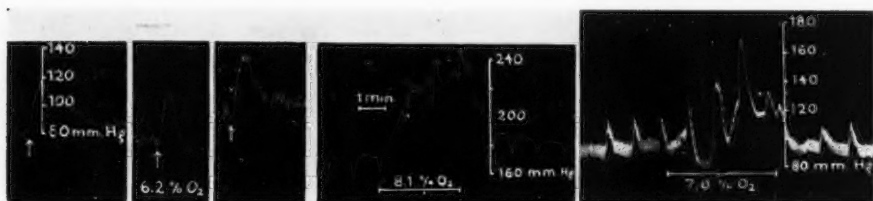


Fig. 3

Fig. 4a

Fig. 4b

Fig. 3. Blood pressure record. Stimulation (as in fig. 2b) of the hypothalamus in a cat with vagi intact, but carotid sinuses denervated. Note the shorter duration of the pressor response during anoxia.

Fig. 4a. Blood pressure record (upper graph). Stimulation of the vasomotor center in the medulla at 1 minute intervals with 60 cycle current at 6 volts for 5 seconds. Note change of depressor to pressor response in anoxia.

Fig. 4b. Blood pressure record (lower graph). Medullary stimulation as in figure 4a, but 3.5 volts were used. Note marked increase in pressor response during anoxia.

Experiments on animals with intact vagi but denervated carotid sinuses show results somewhat intermediate between those obtained in normal and in completely "denervated" animals. Figure 3 shows that anoxia in this case produces no distinct diminution in the pressor response, but causes a marked decrease in the duration of the response.

On the basis of these observations it may be inferred that in the normal animal the hypothalamic centers show a greatly increased excitability during anoxia leading to an increased contraction of the n.m. and to an increased blood pressure response. Whereas the latter depends on the presence of the chemoreceptors, the increased responsiveness of n.m. on hypothalamic stimulation is independent of chemoreceptors.

In order to elicit a contraction of the n.m. from the hypothalamus, it was in

⁴ "Denervated" is used to indicate the denervation of both carotid sinuses and bilateral vagotomy.

general necessary to use relatively strong faradic currents which invariably resulted not only in contractions of the n.m. but also in movements of the extremities, the jaws and other parts of the body. Whereas during anoxia the contractions of the n.m. increased in height these somatic movements greatly decreased in magnitude at the same time. This observation suggests that the somatic centers located in the hypothalamus are depressed during anoxia whereas the sympathetic centers regulating the n.m. show an increased excitability. Systematic observations confirmed this conclusion. By determining the threshold of the movements elicited by stimulation of the hypothalamus, it was found that inhalation of 4.5 per cent oxygen regularly increased the threshold. In one experiment, for example, it was found that the threshold for a movement of the paw was at 6.5 cm. coil distance (c.d.). After five minutes of anoxia the faradic current produced by the inductorium at a c.d. of even 5 cm. could not elicit any movements. Three minutes after the readmission of air the threshold was at 5.5 cm. c.d., $2\frac{1}{2}$ minutes later it was at 6.0 cm. c.d., and after 5 more minutes it had returned to its original level (6.5 cm. c.d.).

II. *The effect of anoxia on the excitability of somatic and autonomic functions of the medulla and the rôle of the buffer nerves.* The effect of anoxia on the contraction of the n.m. elicited by stimulation of the sympathetic medullary centers is similar to that found previously in experiments involving hypothalamic stimulation. The 60 c.p.s. current was used and a threshold contraction was elicited. This reaction remained constant when the stimulation was performed at intervals of one or two minutes. During inhalation of 5.7 per cent oxygen the contraction of the n.m. became maximal. The effect was reversible on readmission of air. The experiments seem to indicate that the sympathetic medullary centers are likewise more excitable in anoxia than under control conditions.

This conclusion is confirmed by experiments in which as indicator of the sympathetic response the erection of the hairs on the back or tail was studied in response to stimulation of the medulla. In one set of experiments the threshold was determined in volts using the General Electric Variac as the stimulating device. During inhalation of 5.7 per cent oxygen the threshold was distinctly lowered. In another series the stimulus was kept constant and the intensity of the reaction was observed. It was found that during anoxia a larger area participated in the piloerection than during inhalation of air. The effects were rapidly reversible on readmission of air.

The reaction of the blood pressure response to medullary stimulation during anoxia is illustrated by figure 4. In the upper part of figure 4 a stimulus of 6 volts was used causing a slight depressor response. During the inhalation of 8.1 per cent oxygen the blood pressure rose and the response to stimulation was converted into a pressor response. Before this happened, however, the depressor response increased temporarily. After readmission of air the stimulation resulted again in a depressor response of slightly lesser magnitude than was observed during the control period prior to the inhalation of low oxygen. The conversion of a depressor into a pressor response was not infrequently found and seems to indicate an increase in the excitability of the vasomotor center in

anoxia, since it was shown in control experiments that a depressor response can easily be changed into a pressor response by slightly increasing the intensity of the stimulus. In other words, anoxia acts as if the intensity of the stimulus had been increased. Apparently, a stimulus applied to a depressor point affects also adjacent pressor points whose threshold has been lowered in anoxia.

The lower graph of figure 4 shows the alteration of a pressor response during inhalation of 7 per cent oxygen. Here again the vasomotor center was stimulated and it is seen that the first three responses are very greatly increased during anoxia. However, the fourth response is actually decreased. This experiment illustrates that although anoxia leads to an increase in the blood pressure reaction on hypothalamic and medullary stimulation in the normal cat the duration of this effect is somewhat variable. In some animals the pressor response remained increased during inhalation of 7 per cent oxygen for as long as 8 minutes and the period of anoxia was not extended any further. In other experiments the period of increased excitability was shorter, as shown in figure 4. It should, however, be

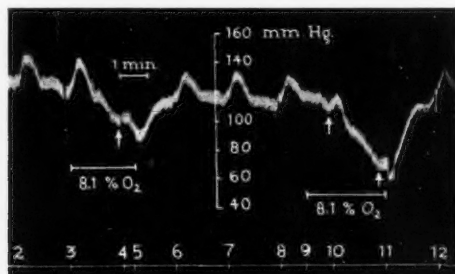


Fig. 5. Blood pressure record. Medullary stimulation at 2 minute intervals with 60 cycle currents at 3 volts for 5 seconds. Bilateral denervation of carotid sinuses, vagi intact. Eight and one-tenth per cent O_2 between 3 and 5 and 9 and 11. The stimulation during anoxia indicated by arrows.

emphasized that the duration of the period in which the pressor responses were increased in anoxia was directly related to the excitability of the central nervous system.

After denervation of the carotid sinuses and bilateral vagotomy entirely different results were obtained. Figure 5 shows that the pressor response which is regularly obtained in the control periods preceding and following the administration of low oxygen almost completely disappears during anoxia. At the same time the blood pressure gradually falls during anoxia. That the decrease or the absence of a pressor response is not due to the fall in blood pressure is shown by the second half of figure 5 in which the stimulus was applied one minute after inhalation of 8.5 per cent oxygen. At that time the blood pressure had not yet fallen but the pressor response was markedly decreased. Further experiments indicated that the same results were obtained in animals in which the carotid sinuses were denervated but the vagi remained intact. Apparently the excitatory impulses from the chemoreceptors of the carotid sinus area are primarily

responsible for the increased excitability of the vasomotor center to direct stimulation during anoxia.

III. *The effect of anoxia on the excitability of somatic and autonomic centers in the spinal cord.* In a series of experiments autonomic and somatic responses were studied by stimulating the dorsal surface of the spinal cord. In some experiments the dura was cut; in others it was left intact. Monopolar as well as bipolar electrodes were used. The various methods did not appreciably alter the response. It was found that stimulation of the posterior columns of the spinal cord between T₂ and T₇ elicited a pilomotor response either on the tail or on the back. During the inhalation of 4.5 per cent to 6.2 per cent oxygen this response was distinctly increased while the stimulus remained the same. Such results were obtained not only in narcotized but also in decerebrate animals. This suggests that the increased response of the autonomic reactions to a standard stimulus applied to the spinal cord is not due to a removal of inhibition from higher parts of the central nervous system but is caused by an increase in the excitability of the autonomic centers of the spinal cord.

Similar results were obtained by stimulating T₁ to T₄ before, during and after inhalation of low oxygen when the contraction of the n.m. was recorded. The results agreed with those previously described; i.e., the contraction of the n.m. increased during the period of anoxia while the stimulation was kept constant. Finally the effect of stimulation of the dorsal columns of the spinal cord on somatic movements was studied and it was found that in contradistinction to the autonomic responses the somatic movements definitely declined during anoxia. Similar results were obtained in decerebrate dogs.

The results appear to be due to changes in the excitability of spinal and not of medullary centers for the following reason. Stimulation of the lower thoracic cord never elicited a contraction of the n.m. although this effect was regularly produced on stimulation of the first three or four thoracic segments. The changes in blood pressure resulting from the stimulation of the cervical spinal cord were slight or nil whereas the same stimulus applied to the lower thoracic cord resulted in very great pressor responses. Furthermore, stimulation of the surface of the spinal cord at the lumbar level failed to evoke reflex movements of the forelegs. Apparently, the conditions of stimulation were such as to affect primarily the stimulated segments.

DISCUSSION. The chief result of our investigation is the fact that whereas autonomic responses (pilomotor and blood pressure response as well as contraction of the n.m.) are increased in anoxia, somatic responses obtained from the same level (experiments on the hypothalamus and the spinal cord) decrease. As far as the blood pressure reaction is concerned, this effect is obtained only in the presence of the buffer nerves. In their absence the vasomotor center reacts similarly to somatic centers inasmuch as the blood pressure response to stimulation markedly declines in anoxia. Since it has been shown that this decline is not due to a fall in blood pressure level it is concluded that the excitability of the medullary vasomotor center is increased in anoxia only when it is under the influence of afferent stimuli from the sino-aortic area. Similar results are ob-

tained when the blood pressure reaction is elicited from the hypothalamus. Apparently not only the tonicity of the vasomotor center depends on the impulses originating in the chemoreceptors (Gellhorn and Lambert) but also its reactivity to direct stimulation and to stimuli coming from supra-medullary regions (hypothalamus) is determined by these afferent impulses.

The contraction of the n.m. was shown to be increased during anoxia on hypothalamic stimulation and this effect was independent of the presence of the carotid sinus nerves. These data suggest that the autonomic centers in the central nervous system are less sensitive to anoxia than are somatic centers. The vasomotor and the respiratory center are intermediate between the somatic centers whose excitability declines in anoxia even in the normal animal and the sympathetic center in the hypothalamus governing the activity of the n.m. which remains in a state of heightened excitability in spite of the absence of the buffer nerves. It is assumed that the increased excitability of the autonomic centers greatly contributes to the resistance of the organism to anoxia. Moreover, experiments which will be reported elsewhere indicate that the greater resistance of the autonomic when compared to that of the somatic centers is not restricted to the conditions of anoxia. The observations of Feldman, Cortell and Gellhorn that anoxia leads to a discharge over both sympathetico-adrenal and vago-insulin systems is in line with this argument and suggests that both divisions of the autonomic nervous system are characterized by a greater resistance to anoxia than is shown by cerebro-spinal centers at similar levels.

SUMMARY

The effect of anoxia produced by inhalation of oxygen-nitrogen mixtures varying between 4.5 per cent and 8.1 per cent oxygen was studied on autonomic and somatic responses elicited by stimulation of hypothalamus, medulla and spinal cord in narcotized cats.

Anoxia increases the contractions of the n.m. produced by stimulation of the hypothalamus and this effect persists after the denervation of the carotid sinuses and bilateral vagotomy. Since anoxia exerts no effect on the contraction of the n.m. elicited by stimulation of the cephalic end of the cervical sympathetic, the increased response to hypothalamic stimulation is attributed to an increased excitability of the hypothalamic sympathetic centers. Somatic movements elicited from the same area, however, decline or disappear under conditions of anoxia.

The blood pressure rise resulting from hypothalamic stimulation increases during anoxia in the normal animal but decreases in the cat deprived of the carotid sinuses and vagi. Similar results are obtained in experiments on direct stimulation of the vasomotor center in the medulla. They suggest that the increased excitability of the vasomotor center to indirect (supramedullary) as well as direct stimuli (applied to the vasomotor center itself) depends on the presence of afferent impulses from the chemoreceptors of the sino-aortic area.

Further studies on the effect of anoxia on the contraction of the n.m. and the erection of hairs elicited by medullary and spinal stimulation indicate an in-

creased excitability of these centers in anoxia. Somatic movements induced by stimulation of the spinal cord decline during anoxia. These results were obtained in narcotized as well as in decerebrate cats.

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THE UTILIZATION OF BLOOD OXYGEN BY THE DISTENDED INTESTINE

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The flow of blood through the dog's small intestine has been shown to be only temporarily reduced when the intestine is distended at pressures below 30 cm. water (Lawson and Chumley, 1940). Some increase of flow above the initial maximum reduction has been found to occur during distention at all pressures below the level of mean arterial pressure (unpublished data). The total flow may thus return, during distention, to its control level or to a level somewhat above or somewhat below its control, depending upon the distention pressure employed and the condition of the intestine. If no change occurs in the percentage distribution of the total flow among the various tissues of the system intestine-mesentery, the recovery of flow during the early portion of the distention period should, if it is complete, ensure adequate circulation of all the tissues of the distended gut. Pappenheimer (1941) has shown, however, that redistribution of flow can occur within an organ independently of changes in total flow, and that as a result, portions of the organ may be denied a full supply of oxygen at rates of total flow which are adequate if equitably distributed.

In the present study an attempt was made to use changes in the consumption of oxygen by the circulated gut during distention as an index to changes in the oxygen supply. Since the blood flow through most organs is considerably in excess of the amount needed to meet the oxygen requirements (Barcroft, 1934), oxygen consumption is independent of flow between fairly wide limits. Confirmation of this for the intestine under our working conditions was obtained in the course of this study (see table 3). It was expected, therefore, that the oxygen consumption of the loop would be unaffected by changes in flow unless flow were reduced, in some portion of the gut, below the minimum required to supply oxygen to that portion. As is shown by our data, this minimum is reached in some part of the gut when flow to the whole loop is reduced to about 20 cc./100 gm./min., or about one-half of its usual rate. These limitations of the method led us to believe that we would have to reduce flow in the undistended loop to a demonstrable minimum for oxygen supply before we could hope to obtain evidence, from a reduction in oxygen consumption, that distention had reduced the flow to any portion of the loop.

The assumption was made, in planning the study, that any reduction in flow

to the tissues of the distended gut would be the direct mechanical effect of the distention on resistance in the tissues. It was assumed that with moderate distentions, the effect would be small, and the flow reduction far from critical for oxygen consumption. We were therefore unprepared for the striking data obtained in preliminary experiments in which no attempt had been made to reduce flow in the resting loop to critical rates for oxygen supply. The data are less surprising if the theoretical effect of low-resistance vascular shunts on flow through the tissues is kept in mind.

METHODS. *Blood flow measurements.* The volume flow of blood through loops of ileum or jejunum was measured in barbitalized dogs. In about half the experiments differential manometry was employed as described in previous reports (Lawson, 1941). In the others venous outflow was measured directly by temporarily diverting the blood flowing from the loop into a measuring bulb, the rise in venous pressure during the collection being not more than 3 cm. blood. By keeping a layer of mineral oil in the collecting bub, this measurement was carried out anaerobically, so that the collected sample was available for oxygen determination as described below. Chlorazol Fast Pink (reprecipitated) was given intravenously in doses of 100 mgm. per kgm. as anticoagulant in the measurement of outflow. As is shown in table 1, values for blood flow and for oxygen consumption do not appear to be significantly different with the two methods.

All of the data reported here were obtained with distentions at an intrainestinal pressure of 30 cm. water, during which recovery of flow is usually not quite complete. The technique of distention was the same as in the earlier reports.

Collection of blood samples and measurement of arterio-venous oxygen difference. Oxygen was determined on 1 cc. samples by the manometric method of Van Slyke and Neill (1924). Arterial samples were drawn under oil from a femoral artery at the beginning of the experiment, at the end, and at intervals of 10 to 15 minutes throughout. At least one arterial sample in each experiment was drawn during distention of the intestine. Abrupt changes in arterial oxygen were not observed, the values obtained either remaining constant or changing progressively at a practically uniform rate throughout the experiment. The values for arterial oxygen were plotted against time, and from the resulting curve arterial oxygen at the time of collection of each venous sample was obtained by interpolation. The method is less dependable than simultaneous measurement of arterial and venous oxygen, but is sufficiently accurate for our purposes, since we demonstrated that our procedures were without effect on arterial oxygen.

Venous samples from the resting (undistended) loop were drawn at least three minutes after termination of a preceding distention period, to permit full recovery. There was usually fairly good agreement between the first and subsequent resting determinations, suggesting that this recovery period was adequate (see table 1). Samples from the distended loop were drawn routinely two minutes after beginning inflation. At this time blood flow has usually reached a state of no further change, following recovery from its initial reduction.

TABLE 1
O₂ consumption of resting innervated intestine

ANIMAL NO.	METHOD	OBSERVATION NO.	A-V O ₂	BLOOD FLOW	O ₂ CONSUMPTION
			cc.	cc./100 g./min.	cc./100 g./min.
1	A	1	9.25	42	3.88
		2	14.24	29	4.86
2	V	1	4.20	65	2.73
		2	2.30	70	1.61
3	V	1	2.05	84	1.72
		2	2.92	75	2.18
		3	1.10	73	0.80
4	V	1	4.66	40	1.86
		2	4.51	36	1.64
5	V	1	4.53	41	1.87
		2	3.01	43	1.41
6	V	1	2.37	68	1.61
		2	2.50	54	1.36
		3	2.18	62	1.36
7	A	1	2.99	50	1.49
8	A	1	1.60	87	1.39
		2	1.10	87	0.96
		3	1.36	73	1.00
9	A	1	2.38	30	0.71
		2	2.72	32	0.87
		3	2.50	30	0.75
10	A	1	2.92	48	1.40
		2	2.39	45	1.07
		3	2.55	43	1.10
11	V	1	1.59	81	1.28
		2	1.95	80	1.72
		3	2.02	73	1.47
12	A	1	3.60	50	1.78
		2	4.80	36	1.74
		3	5.51	26	1.54
13	A	1	1.79	97	1.74
		2	1.80	72	1.30

V = Venous outflow measurement; A = arterial flow by differential manometry.

In obtaining venous samples, admixture with other portal blood was prevented by clamping the vein which drains the loop downstream from a convenient side

branch which was opened for collecting the sample. When flow was measured by differential arterial manometry the sample was drawn into an oiled syringe through a blunt needle tied into the side branch. With the direct measurement of outflow, a bottom outlet from the measuring bulb permitted the sample to be drawn off under oil after its rate of flow into the bulb had been measured.

RESULTS. *The consumption of oxygen by the resting intestine.* Table 1 summarizes the data obtained in 32 determinations on 13 undistended innervated loops. As is shown in the table, most of the rates of oxygen consumption lie between 1 and 2 cc. per 100 grams per minute, with extremes of 4.86 and 0.71 cc. The limits of variation in any single loop during the course of the experiment are considerably less than the limits for the group as a whole, and with only two exceptions lie between +24 and -21 per cent of the mean for the loop. No relationship is apparent in the table between the rate of flow and the rate of oxygen consumption. These rates are similar to those found by Brodie and his colleagues (1910), who also observed a lack of relationship between flow and oxygen consumption, and noted unexplained variations in oxygen consumption of the same order of magnitude. No explanation is available for the large number of loops in this group with rates of flow excessively high as compared with those previously reported (Lawson, 1941). The work was done for the most part during a period of extremely warm weather, on a group of dogs with low cell:plasma ratios and low arterial oxygen.

The resting oxygen consumption of denervated intestine, as shown by 30 determinations on 12 loops whose mesenteric nerves had been sectioned, did not appear to differ significantly from that of intestine with its nerves intact. In this series the variations found on repeated determinations in the same loop were between +19 and -28 per cent of the mean for the loop.

The effect of distention on innervated intestine. In determining the effects of distention, data for the period of distention were always paired with data for the immediately preceding control period. The difference between the control and the distention data in each such pair is shown in figures 1 to 3 as a percentage of the control. In the figures the data are arranged in the order of the change in oxygen consumption, to show relationships between oxygen consumption, blood flow, and the A-V oxygen difference. Figure 1 summarizes, in these terms, the effect of distention (32 trials) on the 13 innervated loops whose control data were given in table 1. The reduction in oxygen consumption which was observed in all except 5 of the 32 trials is usually due, as the figure shows, to a decrease in the A-V oxygen difference, and bears no constant relationship to the change in blood flow. Some of the more striking reductions in oxygen consumption were associated with no change, or with an increase in total blood flow.

The effect of distention on cocaineized intestine. Since the application of 1 per cent cocaine hydrochloride solution to the mucosa of the loop prevents recovery of blood flow during distention (Lawson and Chumley, 1940), it seemed probable that a study of the cocaineized intestine would throw light on the relationship between the recovery of total flow and the change in oxygen consumption during distention. If the recovery of total flow through the distended loop is brought

about by the opening of nutrient vessels for the tissues, abolishing the recovery mechanism with cocaine should increase the flow deficit in the tissues, and augment the reduction in blood oxygen utilization. If, on the contrary, the recovery of total flow is due to the opening of vascular by-passes, its abolition by cocaine should augment neither the flow deficit in the tissues nor the reduction in oxygen utilization.

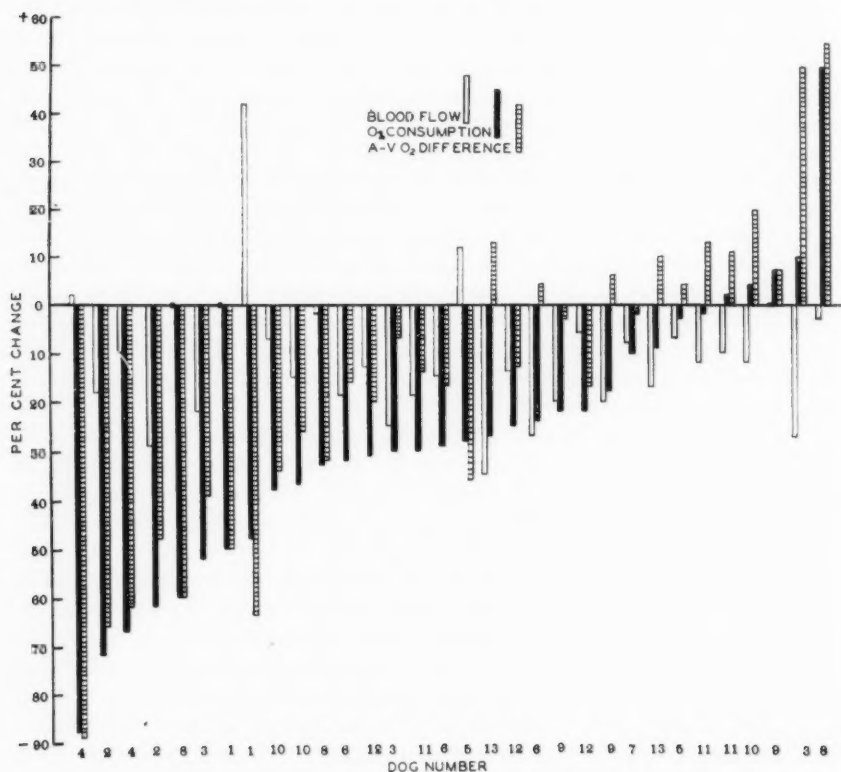


Fig. 1. The effect of distention on innervated, untreated intestine. See text for explanation.

Some of the loops shown in figure 1 were accordingly treated with cocaine, and subjected to periods of distention as above. The paradoxical effect of cocaine is illustrated in the form of a protocol for one of these experiments in table 2. As is shown in the table, despite the fact that distention caused a greater reduction in total blood flow than before cocaine treatment, it no longer reduced oxygen consumption, the A-V oxygen difference approximately compensating for the reduced flow. Data for all the cocaine-treated loops are summarized in figure 2. In every case the arterio-venous oxygen difference was increased during the distention. The decrease in oxygen consumption which was shown

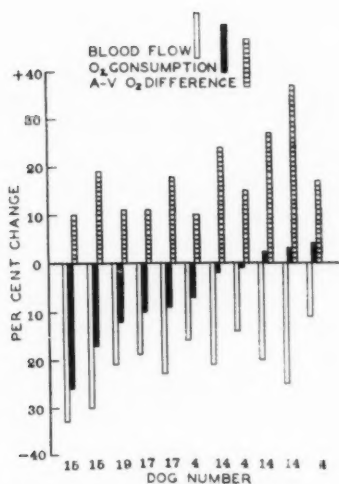


Fig. 2. The effect of distention on cocaine-intestine. See text for explanation.

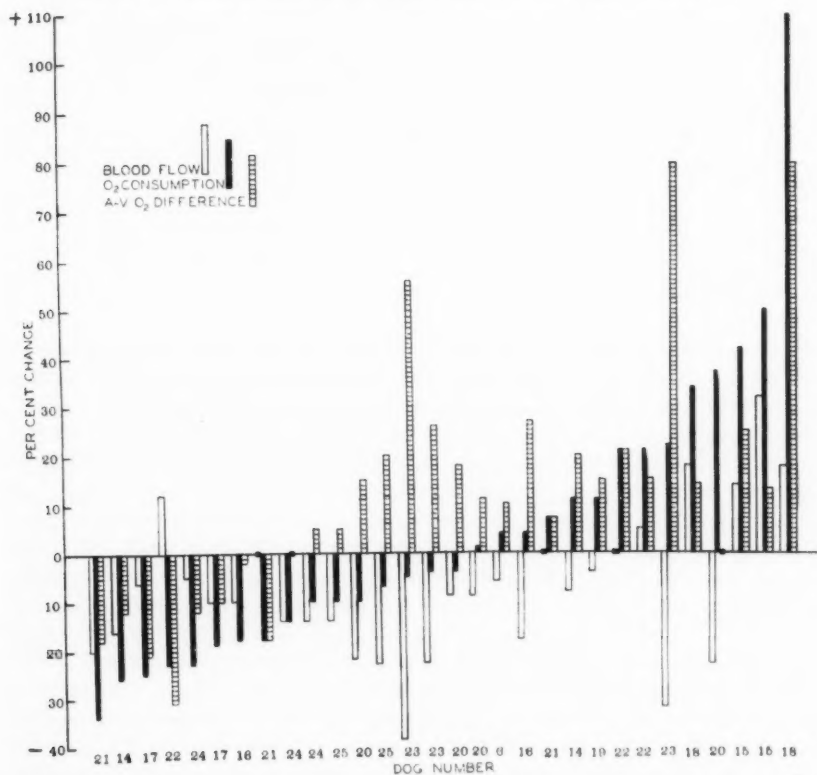


Fig. 3. The effect of distention on intestine after section of mesenteric nerves. See text for explanation.

by some of the loops is small in comparison with the decrease shown by the majority of the untreated loops. It is roughly proportional to the reduction in total blood flow, and inversely proportional to the increase in the A-V oxygen difference.

The effect of distention after section of the mesenteric nerves. In all of the previous studies the effect of distention on total flow through the intestine has been unmodified by section of the mesenteric nerves (Lawson and Chumley, 1940; Lawson, 1941). Figure 3 summarizes our data on the effect of distention on oxygen consumption in denervated loops. In about half the trials oxygen consumption was decreased during distention, and in about half increased. The reductions observed do not exceed the limits of variation (-28 per cent of the mean for the loop) shown by these same loops when undistended, and cannot, therefore, be attributed to the distention. Some of the increases do, however,

TABLE 2
The effect of distention on an innervated loop after cocaineization of the mucosa

OBSERVATION	BLOOD FLOW			A-V O ₂ DIFFERENCE			OXYGEN UTILIZATION		
	Cc./100 g./min.		Per cent change	Cc.		Per cent change	Cc./100 g./min.		Per cent change
	Cont.	Dist.		Cont.	Dist.		Cont.	Dist.	
Untreated									
1	39.9	36.0	-10	4.66	1.70	-61.4	1.86	0.61	-67.2
2	36.3	37.2	+2.2	4.51	0.50	-89.0	1.64	0.19	-88.4
Cocainized									
1	35.4	30.3	-14.4	4.22	4.85	+14.9	1.49	1.47	-1.3
2	31.5	26.4	-16.1	5.28	5.85	+10.8	1.66	1.54	-7.2
3	28.8	25.5	-11.5	5.76	6.75	+17.2	1.66	1.72	+3.6

Cont. = control; Dist. = distended.

exceed the observed limits of random variation ($+19$ per cent), and are probably significant. These data are interpreted as showing that distention was usually without effect on oxygen consumption in the denervated loop, but occasionally caused an increase. The effect of distention on the A-V oxygen difference was strikingly different in the denervated and the innervated group. Whereas in innervated loops the A-V oxygen difference was usually decreased (fig. 1), in denervated loops it was usually increased (fig. 3). At these distending pressures, an increase in total blood flow over the control was observed more frequently in the denervated than in the innervated group, as has already been reported, but in both groups the usual effect was a moderate flow reduction.

An effort was made in three experiments to determine whether the failure of distention to reduce oxygen consumption in the denervated intestine is due to the fact that the resting volume of flow is larger (Lawson, 1941), and therefore more greatly in excess of the requirements of the tissues. Vascular shunts could open under these conditions and divert blood from the tissues without reducing flow through the tissues to critical levels for oxygen supply. Table 3 presents,

in the form of a protocol for one of these experiments, the data obtained on distention of a denervated loop after its resting flow had been reduced to critical levels by application of a Goldblatt clamp to its artery in the mesentery. As is shown in the protocol, resting oxygen consumption was not reduced until arterial compression had reduced the total flow to about 20 cc./100 gm./min. Distention at the higher rates of flow either increased or did not change the A-V oxygen difference and the oxygen consumption. At the lower rates of flow, distention reduced both the A-V oxygen difference and the oxygen consumption. Similar data were obtained in the other two dogs, on the minimum rates of flow required for resting oxygen supply, and on the effect of distention at various flow levels. In none of them, however, was it possible to obtain such marked reductions in oxygen consumption during distention as were observed in some of the innervated loops.

TABLE 3

The effect of distention on a denervated loop with its resting flow reduced (Goldblatt clamp on mesenteric artery)

OBSERVATION	BLOOD FLOW			A-V O ₂ DIFFERENCE			OXYGEN UTILIZATION		
	Cc./100 g./min.		Per cent change	Cc.		Per cent change	Cc./100 g./min.		Per cent change
	Cont.	Dist.		Cont.	Dist.		Cont.	Dist.	
1	92.4	89.6	-3.3	1.65	1.90	+15.2	1.53	1.70	+11.1
Artery constricted									
1	47.2	46.0	-2.5	3.30	3.40	+3.3	1.56	1.56	±0.0
Artery further constricted									
1	20.8	21.2	+4.6	5.95	5.30	-10.9	1.23	1.12	-9.0
2	18.4	16.0	-13.0	6.90	5.57	-19.3	1.27	0.89	-30.0

Cont. = control; Dist. = distended.

DISCUSSION. With an adequate supply of oxygen for all parts of the intestine moderate distention would be expected to increase oxygen consumption since it demonstrably increases both muscular and secretory activity. The possibility, however, that even such drastic reductions as are reported here are due to reduced oxygen requirements during distention, cannot be categorically dismissed without additional data. We have provisionally taken the data on the innervated intestine to mean that when the loop is distended, the flow of blood is diverted from channels in which oxygen loss is high into channels in which it is low. That the latter are functional, if not anatomical arteriovenous anastomoses (Spanner, 1932) is suggested by the fact that blood passing through them during distention may lose almost none of its oxygen.

Cocaine, which has been shown in previous reports to prevent recovery of total flow during distention, also has the property of preventing a reduction in oxygen consumption. These data suggest that the mechanism which deprives the active tissues of oxygen during distention is the mechanism which restores total flow. The failure of distention at these pressures to reduce oxygen con-

sumption in the cocaineized loop suggests, further, that in the absence of the short-circuiting action of the shunts, flow through the tissues of the gut is not seriously impeded by moderate distention.

Our failure to obtain satisfactory evidence for the operation of vascular bypasses during distention of the decentralized intestine is tentatively attributed to the larger total volume of flow, and not to the interruption of reflex arcs required for opening of the shunts. Our data showing that the decentralized intestine with its flow restricted resembles the innervated gut are not, however, altogether convincing, since drastic curtailment in oxygen consumption during distention, to match the more striking cases in the innervated series, was not obtained. The applicability of these data to the problem of intestinal obstruction, in which ischemic damage may be done by the development of pressure within the obstructed intestine, should be investigated. It has been shown that decentralization of obstructed or distended intestinal loops prolongs the period of survival (Herrin and Meek, 1933; Antonicic and Lawson, 1941).

The observation that cocaine abolishes deflation hyperemia, but does not abolish the hyperemia which follows a period of arterial occlusion was recently offered as evidence against the occurrence of masked ischemia in the inflated intestine (Lawson, 1941). The present data, if our interpretation of them is correct, show that ischemia does occur in portions of the distended innervated loop, and is masked by a compensatory increase in flow in other portions. Cocaine prevents the occurrence of such ischemia, hence cannot be used to differentiate deflation hyperemia from reactive hyperemia, as in the earlier report.

SUMMARY AND CONCLUSIONS

During moderate distention (distending pressure 30 cm. water) of the barbitalized dog's ileum or jejunum there is usually a marked rise in the oxygen content of venous blood returning from the loop. The arterio-venous oxygen difference may fall, for this reason, to one-half or less of its undistended control value. The volume flow of blood, after recovery from its initial brief period of reduction, is usually slightly less than during the control, but is sometimes unchanged, or even increased. There appears to be no relationship between the direction or amount of change in blood flow, and the direction or amount of change in the arterio-venous oxygen difference. The oxygen consumption of the intestine, calculated as the product of flow by A-V oxygen difference, is almost always reduced.

After treatment of the intestine with cocaine, the oxygen content of mesenteric venous blood is always reduced during distention. There is little or no recovery of blood flow, so that throughout the distention flow is considerably more reduced than in the untreated intestine. Oxygen consumption, calculated as the product of flow by A-V oxygen difference, is either unchanged, or reduced slightly in proportion to the flow reduction.

Distention of the untreated intestine after section of its mesenteric nerves has no consistent effect on either the oxygen content of mesenteric venous blood or

the calculated oxygen consumption of the intestine. The effect on total blood flow through the intestine does not appear to differ from that in the intact gut except that increases in flow over the control during the distention period are more frequent. In a small number of cases the calculated oxygen consumption was significantly increased during distention, over the control. After flow to the decentralized intestine had been reduced by compressing its artery, distention consistently reduced the arterio-venous oxygen difference and the calculated oxygen consumption, but not so greatly as in the intact intestine.

These data are offered as incomplete evidence for the opening of low-resistance vascular shunts in the distended intestine, which short-circuit some of the tissues of the gut wall, depriving them of their oxygen supply, and which at the same time provide for the maintenance of an undiminished total volume flow of blood. Cocaine appears to prevent the opening of the shunts. Failure to demonstrate, by these methods, the operation of the shunt mechanism in the intestine after section of the mesenteric nerves can be explained by assuming that flow to the tissues of the denervated loop is greatly in excess of the minimum rate required for oxygen supply.

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RELATIONSHIP BETWEEN THE PARATHYROID AND THE GASTRIC GLANDS IN THE DOG¹

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The literature dealing with the relation of the parathyroid glands to gastric secretion has been reviewed in an earlier communication by Babkin, Komarov and Komarov (3). Although many important facts were established by previous investigators, they are in some respects open to criticism. Thus few investigations were carried out in which more than one type of gastric preparation was used, and the stimuli employed for the production of gastric secretion were limited to either meat or histamine. When Pavlov-pouches were used, evidence was not always presented to prove that the pouches were innervated. Few determinations were made of the peptic power of the gastric juice. Although the parathyroid hormone, when injected subcutaneously, does not exert its maximum effect on the serum calcium until at least 12 hours after its administration, some experiments were performed in which the hormone and the gastric stimulus were given simultaneously. Therefore it seemed desirable to re-investigate the whole problem, using more adequate methods.

The purpose of the present investigation was to study the effect of the parathyroid hormone and parathyroidectomy on gastric secretion provoked by various stimuli in innervated and denervated gastric pouches in dogs. Preliminary reports of this work have appeared (2, 9).

METHODS. Three types of experimental preparation were used: Pavlov-pouch, Heidenhain-pouch and esophagotomy with a gastric fistula. The Pavlov and Heidenhain pouches were provided with a narrow orifice which acted as a valve and prevented the escape of gastric juice from the pouch. A soft rubber catheter (10 Fr.) was used to withdraw the secretion from the pouch. Ulceration of the skin around the orifice of the fistula never occurred with this type of preparation. The dogs were kept on a standard diet and care was taken to replace the hydrochloric acid and salt lost with the gastric juice during the experiments.

We were faced with the problem of keeping the dogs alive and in a state of

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good nutrition after parathyroidectomy. It was decided that Parathormone would not be a suitable substitute for the secretion of the parathyroid glands because of its long latent period and its after-effects on gastric secretion; therefore, calcium lactate was given to the animals with their daily food. Sterile 5 per cent calcium lactate was prepared for emergency use and injected intravenously when necessary. The animals were observed at frequent intervals during the day, and the time between the last observation at night and the first on the following morning never exceeded seven hours. With these precautions it was possible to keep the dogs alive and in good condition for as long as three months after complete thyroparathyroidectomy.

The free and total acidities of the gastric juice were determined by titration with 0.02 N NaOH, Töpfer's reagent and phenolphthalein respectively being used as indicators. The peptic activity was determined by Nirenstein and Schiff's modification of Mett's method. The Volhard-Harvey method, as described by Peters and Van Slyke (8), was used for the determination of chloride.

The concentration of serum calcium was determined by the method described by Clark and Collip (6). The parathyroid hormone preparation used in these experiments was Parathormone (Eli Lilly Co.).

RESULTS. Effect of Parathormone. Pavlov-pouch dog "S". The gastric secretory responses to various test-meals (bread, milk or meat) and to subcutaneous injection of 0.75 mgm. histamine phosphate (Parke, Davis & Co.) were determined in dog "S", male, weighing 22.5 kgm. by the following procedure. The test-meal was given about 18 hours *post cibum*, and the secretion was removed hourly, the volume of each sample being recorded and the juice analyzed for acidity, pepsin and chloride. The duration of the collection period was 8 hours. When histamine was administered, the juice was collected at intervals of 15 minutes for one hour. Precautions were taken in all these experiments to prevent the provocation of secretion by any outside stimulus.

Table 1 summarizes the results obtained with dog "S". Fifty units Parathormone were injected subcutaneously 12 to 13 hours before the start of the experiment. The average increase in serum calcium was 1.4 mgm. per cent. In every case, the injection of Parathormone caused a diminution in the total volume of secretion and increased the concentration of pepsin. The acidities were decreased in the experiments with bread and milk. The concentration of chloride was slightly less than in the control experiments. In addition, the curve of the hourly rates of secretion was distorted. For example, the initial high rate of secretion in response to a meal of bread was depressed and a new peak was reached at about the fourth hour. Although the serum calcium had returned to normal levels within 48 hours after the injection of Parathormone, the initial control values for the feeding experiments were not regained for as long as six weeks. The results of these experiments are expressed as "Post-Parathormone" in table 1.

Dog "D" with esophagotomy and a gastric fistula. The effect of injection of Parathormone on the gastric secretory response to sham-feeding, to intra-

venous injection of 5.0 units insulin (Connaught Laboratories, Toronto) and to subcutaneous injection of 0.75 mgm. histamine phosphate were studied in dog "D", male, weighing 24 kgm.

TABLE 1
Effect of Parathormone on Pavlov-pouch dog "S"

STIMULUS	TYPE	NUM- BER OF EXPERI- MENTS	AVERAGE					
			Total volume	Free acid	Total acid	Cl	Pepsin	Total pepsin
			cc.	m.eq./l.	m.eq./l.	m.eq./l.	Mett units	Mett units
200 grams bread	Control	3	20.3	82.0	109.0	160	220.0	5,370
	Parathormone	2	8.8	48.8	82.5	159	472.0	4,020
	Post-Parathormone	4	13.6	80.2	117.0	160	217.0	3,470
400 cc. milk	Control	3	18.1	77.9	108.0	162	133.0	1,850
	Parathormone	2	6.4	30.3	77.5	160	359.0	1,650
	Post-Parathormone	3	10.9	57.9	103.0	162	138.0	1,680
300 grams meat	Control	3	36.5	100.0	131.0	162	89.3	3,400
	Parathormone	3	21.3	98.2	138.0	161	158.0	2,940
	Post-Parathormone	3	28.1	105.0	143.0	161	152.0	3,820
0.75 mgm. histamine	Control	3	15.1	109.0	137.0	162	60.7	779
	Parathormone	2	11.6	113.0	142.0	161	118.0	1,370
	Post-Parathormone	3	15.7	111.0	143.0	163	75.6	1,120

TABLE 2
Effect of Parathormone on dog "D"

STIMULUS	TYPE	NUM- BER OF EXPERI- MENTS	AVERAGE					
			Total volume	Free acid	Total acid	Cl	Pepsin	Total pepsin
			cc.	m.eq./l.	m.eq./l.	m.eq./l.	Mett units	Mett units
Sham-feed- ing	Control	3	200.0	120.0	148	163	122.0	20,830
	Parathormone	2	115.0	118.0	149	162	186.0	19,400
	Post-Parathormone	2	208.0	112.0	147	164	92.2	15,600
5.0 units insulin	Control	3	159.0	114.0	144	164	185.0	26,400
	Parathormone	2	39.5	98.9	127	162	107.0	3,810
	Post-Parathormone	3	133.0	118.0	146	163	169.0	23,800
0.75 mgm. histamine	Control	3	68.2	114.0	136	162	69.8	4,220
	Parathormone	2	33.5	108.0	134	163	75.7	2,270
	Post-Parathormone	3	64.5	118.0	142	163	89.8	5,330

The results of these experiments are shown in table 2. They are similar to those obtained with the Pavlov-pouch dog in that Parathormone lowered the total volume of secretion. However, with the exception of the sham-feeding

experiments there was no increase in the concentration of pepsin following the administration of Parathormone. The after-effect of the hormone was not observed in this animal. The average increase of the serum calcium after the injection of 50 units Parathormone was 2.1 mgm. per cent.

Heidenhain-pouch dog "M". An attempt was made to obtain a standard response to various test meals in dog "M", female, weighing 12.5 kgm., but the volume of gastric secretion was so small that this project had to be abandoned. The procedure finally adopted was to keep the animal on a standard diet and to collect and analyse the entire volume of gastric juice secreted during a 24-hour period. This was possible because of the sphincter in the opening of the fistula, which prevented the escape of fluid from the pouch. The gastric juice was drained from the pouch twice daily. The data collected during a period following Parathormone administration are shown in table 3. The volume of the secretion, the free and total acidities, and the concentration of chloride were increased after the hormone had been given. There was no significant change

TABLE 3
Effect of Parathormone on Heidenhain-pouch dog "M"

DATE	TOTAL VOLUME	FREE ACID	TOTAL ACID	Cl	PEPSIN	REMARKS
	cc.	m.eq./l.	m.eq./l.	m.eq./l.	Mett units	
2/27/40-3/2/40	26	8.4	22.4	159	72.6	Serum Ca 11.7 mgm. %
3/ 3/40	27	9.4	23.0	160	57.8	
3/ 4/40	23	6.3	16.9	161	64.0	25 U. Parathormone
3/ 5/40	37	43.8	66.1	163	84.6	Serum Ca 14.0 mgm. %
3/ 6/40	48	56.7	78.0	164	70.6	Serum Ca 12.1 mgm. %
3/ 7/40	61	84.2	109.0	163	51.8	
3/ 8/40	57	61.6	80.0	161	77.4	Serum Ca 11.9 mgm. %
3/ 9/40	27	20.9	32.7	161	100.0	
3/10/40	24	12.5	28.2	158	84.6	Serum Ca 11.8 mgm. %
3/11/40	21	7.3	23.3	159	100.0	

in the peptic activity of the juice. The maximum effect on the volume of gastric secretion did not take place on the day following the administration of the hormone and while the serum calcium was at its highest level, but occurred several days after the injection, at which time the calcium concentration had returned to normal levels.

The hormone was given again in an experiment not shown in table 3; the concentration of serum calcium was increased but the volume of gastric secretion was not much above control values. The third time the hormone was administered there was no rise in either the serum calcium or the volume of secretion. Therefore, since Parathormone was no longer effective, it was decided to study the effect of irradiated ergosterol. Twenty thousand units ergosterol per kilogram per day were given orally for eight days. As a result there was an increase of 0.5 mgm. per cent in the serum calcium with little change in the volume of secretion. The dose of ergosterol was then increased, 40,000 units per kgm. being given daily for four days. The animal now refused to eat, but the volume

of secretion was increased by more than 100 per cent and the juice had all the characteristics of that secreted after the administration of Parathormone. The serum calcium was increased by about 2.0 mgm. per cent and the effect on the gastric secretion persisted for more than two weeks after the ergosterol had been discontinued.

Effect of Parathyroidectomy. Dogs with esophagotomy and a gastric fistula. Dog "D" was the first of our experimental animals to be parathyroidectomized. One lobe of the thyroid and two parathyroid glands were removed on December 10, 1940. The concentration of serum calcium at the time of operation was 12.0 mgm. per cent. The serum calcium and the gastric secretory responses remained unchanged for three weeks after the operation. The third parathyroid gland was then removed and within five days the calcium concentration fell to 10.2 mgm. per cent. Ten days after this operation, however, the calcium

TABLE 4
Effect of parathyroidectomy on dog "D"

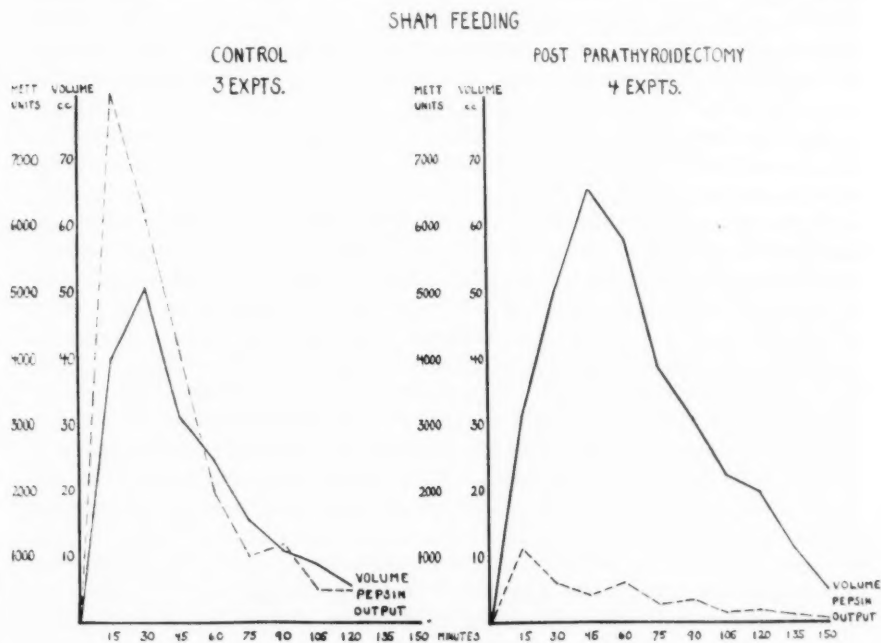
STIMULUS	TYPE	NUM- BER OF EXPERI- MENTS	AVERAGE						
			Total volume	Free acid	Total acid	Cl	Pepsin	Total pepsin	Serum calcium
			cc.	m.eq./l.	m.eq./l.	m.eq./l.	Mett units	Mett units	mgm. per cent
Sham-feeding	Control	3	190	124	149	163	136.0	21,400	12.1
	Post-operative	7	333	131	154	165	10.4	3,490	7.5
	I.V. calcium	2	161	119	140	164	107.0	17,200	10.9
5.0 units in- sulin	Control	3	166	117	145	164	120.0	19,100	12.3
	Post-operative	2	316	117	148	165	9.3	2,840	7.3
	I.V. calcium	1	140	110	143	163	18.0	2,560	10.1
0.75 mgm. histamine	Control	3	71	123	147	163	73.3	5,370	12.2
	Post-operative	6	129	123	150	164	6.1	761	7.1
	I.V. calcium	2	66	114	142	163	13.0	1,400	11.3

had returned to normal levels. The remaining thyroid and parathyroid tissue was removed on January 20, 1941. Five days later the animal's first tetanic attack occurred. The results of the experiments which were performed after the onset of tetany are shown in table 4.

Thyroparathyroidectomy increased the duration and the total volume of gastric secretion. The diminution in the peptic activity of the juice was so marked that although the volume of secretion was increased by as much as 100 per cent in some experiments, the total output of pepsin was less than in the control experiments. Five milligrams calcium (as calcium lactate) per kilogram body weight were given intravenously 45 minutes before the start of the experimental period. This did not produce any salivation and did not stimulate gastric secretion. The volume of the gastric secretory response of the parathyroidectomized animal was diminished by the injection of calcium lactate. The administration of an equivalent amount of lactate (as sodium lactate) had no

effect on the secretory response. The administration of Parathormone acted in a manner similar to that of calcium lactate. To exclude the possible influence of hypothyroidism on these results, 1.0 mgm. thyroxine was injected subcutaneously. The heart-rate thereupon increased from 62 to about 110 within a few days. The experiments performed during the week following the injection of thyroxine had all the characteristics of the previous experiments. This would indicate that the changes in the gastric secretory response were produced by parathyroidectomy and not by thyroidectomy.

In addition to the great increase in the volume of the secretion and the diminution in the output of pepsin following parathyroidectomy, the curve of the rate



of secretion was altered. In the control sham-feeding experiments the peak of the secretion was reached in 30 minutes, whereas after parathyroidectomy the peak was attained at the end of 45 minutes. This is shown in figure 1. These observations were confirmed by experiments performed on dog "P", male, weighing about 10 kgm. This animal was completely thyroparathyroidectomized in one stage. The volume of the gastric secretion in response to sham-feeding was increased by at least 50 per cent and that in response to histamine by 80 per cent. In both types of experiment the peptic activity of the gastric juice was of the same order as that of dog "D" after thyroparathyroidectomy.

Heidenhain-pouch dog "M". This dog differed from dog "D" in that the

removal of one thyroid and two parathyroids caused a slight drop in the concentration of the serum calcium and an increase in the volume and acidity of the gastric juice secreted during 24 hours on a standard diet. The removal of the third parathyroid further decreased the serum calcium and increased the volume and acidity of the gastric secretion. After about one month the serum calcium began to increase and this was reflected in the volume and composition of the gastric juice. Complete thyroparathyroidectomy resulted in a volume of secretion approximately double that of the initial control period. The concentration of pepsin was diminished as in the case of the other thyroparathyroidectomized animals. The dog had frequent tetanic seizures after the last operation.

Pavlov-pouch dog "H". Dog "H", male, weighing 14 kgm., was completely thyroparathyroidectomized in one stage. The volume of secretion provoked by meat, milk and histamine was increased 45 to 80 per cent following parathyroidectomy. The peptic power of the juice was reduced to less than 20 per cent of the control experiments. This reduction in the pepsin concentration was so marked that, despite the increase in the volume of the secretion, the total output of pepsin was always less than in the control experiments.

DISCUSSION. *Hypercalcemia.* These results indicate the great difference between the responses of innervated and denervated gastric pouches under conditions of hypercalcemia. The rise in serum calcium decreases the volume of secretion of the former type of pouch and increases that of the latter type. This may possibly explain the conflicting results reported by other workers. Although they described their fistulae as Pavlov-pouches, these may in reality have been denervated pouches. Austin and Matthews (1) were of the opinion that the parathyroid hormone did not influence the volume of the gastric secretion provoked by histamine if the water balance of the experimental animal was maintained. They were able to show that dehydration took place after the administration of the hormone. This was to have been expected in their experiments because the blood calcium was raised far above physiological levels. Our experiments were performed at more physiological concentrations of calcium and evidence obtained from experiments performed on dog "S" was presented to prove that dehydration did not occur (2).

We obtained no experimental evidence which would explain the after-effect of Parathormone on gastric secretion as observed in the Pavlov-pouch dog, and also the delayed increase of secretion in the Heidenhain-pouch dog. After the latter animal proved to be refractory to the hormone, it was still possible to increase the serum calcium by the administration of large doses of irradiated ergosterol; this, however, cannot be taken as proof that ergosterol and the parathyroid hormone act in different manners.

The experiments on the dog with esophagotomy and a gastric fistula confirm the observations of Babkin, Komarov and Komarov (3) with regard to the effect of the hormone on the volume of the gastric secretion. The diminution in the peptic activity which they noted was not observed in our experiments. This may be attributed to the difference in the methods of administration of

the parathyroid hormone. Their animal was subjected to frequent injections of large doses of the hormone, which damaged the kidneys and altered the chloride content of the blood. We were unable to find any change in either the chloride or the urea concentration in the blood of the animal used in this study. Another interesting fact which was observed, and which has already been reported by Babkin (2), was that the injection of very small amounts of Parathormone decreased the serum calcium and increased the volume of the gastric secretion. This effect is the direct opposite of that observed after the administration of the hormone in amounts sufficient to raise the serum calcium.

This study of the effect of the parathyroid hormone on gastric secretion removes much of the confusion that has existed in the literature, for it has shown that in the relationship between the parathyroid and the gastric glands at least three important factors are involved: 1, the amount and manner of introduction of the stimulus which affects the serum calcium; 2, the type of gastric preparation used; 3, the method of stimulating gastric secretion.

Hypocalcemia. The decision to subject dogs "P" and "H" to complete thyro-parathyroidectomy without first attempting a partial parathyroidectomy proved to be unfortunate. These animals did not survive the operation as long as those on whom partial parathyroidectomy had been first performed. It appeared that the partial removal of the glands permitted some bodily adjustment that enabled the animals to survive subsequent total thyro-parathyroidectomy with the aid of occasional intravenous injections of calcium lactate.

One might anticipate that hypocalcemia would have the opposite effect of hypercalcemia on the secretion of the innervated pouch, but the similarity in the respective effects of these conditions of the blood on the denervated pouch was unexpected. Since the gastric secretion in the Heidenhain-pouch dog was due only to chemical stimulation, an explanation of the results might be found in a consideration of this factor. Carlson (5) reported that the emptying time of the stomach was delayed after parathyroidectomy. This would cause the chemical phase of gastric secretion to be augmented, which might serve as an explanation of our results. However, the effect of parathyroidectomy as observed in the dog with esophagotomy and a gastric fistula is not consistent with this view. With this type of preparation there was no chemical phase of gastric secretion, yet the curve of the secretion in response to sham-feeding (see fig. 1) was altered after parathyroidectomy, since the peak of the secretion was reached later than in the normal animal. It would seem, therefore, that some factor was involved other than, or in addition to, the delay in the emptying time of the stomach.

Dog "D" was sacrificed in the following manner. An acute experiment was performed in which a small piece was cut from the anterior wall of the corpus of the stomach. The vagi were then stimulated rhythmically with an induction current for six hours. The total volume of gastric secretion during this period was 41 cc.—a smaller amount than is usually produced. Another piece was then cut from the posterior wall of the corpus. Both pieces of mucosa were fixed and stained for pepsinogen granules according to Bowie's method (4).

The number of granules was less than in the normal animal and only the cells in the lower half of each gland contained granules. The decrease in the number of granules after stimulation of the vagi was also less than normal. Histological examination of the gastric glands of dogs "P", "H" and "M" also showed less than the normal amount of pepsinogen granules. It may be that parathyroidectomy inhibited the formation of pepsin or diminished the rate at which pepsin was secreted, or produced both these effects. The fact that the amount of pepsin was decreased in the secretion from both the innervated and the denervated type of gastric pouch would indicate that this effect of parathyroidectomy was not mediated through any nervous mechanism.

The continuous spontaneous secretion following parathyroidectomy reported by Lebedinskaia (7) was not observed in our dogs.

SUMMARY

1. The administration of the parathyroid hormone (Parathormone) in amounts sufficient to increase the concentration of calcium in the serum approximately 2.0 mgm. per cent had the following effects on gastric secretion:

a. Pavlov-pouch dog. The volume and acidity were decreased and the concentration of pepsin was increased in response to various test meals and histamine. This inhibition persisted after the serum calcium had returned to normal levels.

b. Dog with esophagotomy and a gastric fistula. The volume of the response to sham-feeding, insulin and histamine was decreased. The concentration of pepsin was increased in the sham-feeding experiments. There was no after-effect such as was observed with the Pavlov-pouch dog.

c. Heidenhain-pouch dog. The volume, acidity and chloride concentration were increased without affecting the concentration of pepsin. The maximum effect on gastric secretion took place several days after the administration of the hormone. Irradiated ergosterol acted in a manner similar to that of Parathormone.

2. The effects of thyroparathyroidectomy were as follows:

a. The volume of secretion was increased and the concentration of pepsin was decreased in the gastric secretory response of the Pavlov-pouch dog, the Heidenhain-pouch dog and the dogs with esophagotomy and a gastric fistula.

b. The intravenous injection of calcium lactate or the subcutaneous administration of Parathormone decreased the hypersecretion following parathyroidectomy.

c. The administration of thyroxine did not affect the gastric secretory response.

d. Histological examination of the gastric glands showed a diminution in the number and distribution of pepsinogen granules.

I wish to express my appreciation of the invaluable advice and criticism which I have received from Prof. B. P. Babkin who directed this work. I also wish to thank Dr. S. A. Komarov for his most generous coöperation.

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A STUDY WITH RADIOACTIVE PHOSPHORUS OF THE PERMEABILITY OF THE RAT PLACENTA TO PHOSPHOLIPID¹

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Studies on permeability of the placenta have been made by many workers using various methods. The majority of this literature is reviewed by Needham (1931). With the production of radioactive isotopes a new tool was added for these studies. First to use them in placental studies was Flexner and his associates who have used radioactive sodium in the form of NaCl in experiments on different types of placentae, among which may be cited the work on the rat (1939, 1941).

In a recent paper Huggett (1941) has reviewed the general subject of the nutrition of the fetus. In this review the placental transfer of lipids has been discussed quite adequately. That there has been and still is considerable disagreement concerning the manner in which lipids reach the fetus is evident from this report. Most of the data forming the bases for this reported work are derived from the allantoic placenta alone, but there are several reasons for believing that the yolk-sac placenta of rodents should be considered in all placental studies as an important adjunct, or perhaps even a separate entity in the general physiology of the fetus.

The present study was begun in an effort to cast further light on the problem of the permeability of the placenta of the white rat to phospholipid.

METHODS. In the present study a comparison was made of the amount of radioactive phospholipid that could be recovered from two groups of fetuses after a limited time interval following intravenous injection of the mothers of the one group with inorganic P³² and the mothers of the second group with tagged phospholipid. This is essentially a measurement of the amount of radioactive phospholipid in the fetuses at a certain time, and not a direct measurement of placental transfer, but the permeability of the placenta can be inferred indirectly by this method.

Haven and Bale (1939) studied the fate of tagged phospholipid injected intravenously into the rat and showed that it increases in the liver and spleen up to 2.5 hours. From then on mobilization occurs and the phospholipid fraction of these organs decreases as activity increases in the intestinal tract, bones and excreta. In view of these findings and those of Perlman, Ruben and Chai-

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koff (1938) on the synthesis of tagged phospholipid following injection of inorganic P^{32} , it was decided in the present study to extract the fetal phospholipid before the general mobilization of the injected phospholipid occurred in the pregnant mothers. This mobilization may be a simple movement of the originally injected material or it may be a movement of resynthesized lipid, or both. The extracting of the fetal phospholipid was carried out in the present work 2 hours after injection into the maternal circulation in the belief that in this time there would be but a small amount of resynthesis and mobilization in the maternal organism.

Radioactive phosphorus was prepared in the pressure electrostatic generator of the Department of Physics and made ready for injection by the Department of Chemistry of the University of Wisconsin. The material was present then as very small amounts of neutral phosphate. Phospholipid containing P^{32} synthesized into the molecule was extracted from the pooled livers, intestines, kidneys and spleens of adult white rats which had been previously fed P^{32} . Each of these rats received 1 or 2 cc. of the phosphorus solution of a known radioactivity by stomach tube, followed immediately by 1 cc. of cod liver oil. They were sacrificed after 6 hours and the extraction and isolation carried out after the method of Bloor (1929). That tagged phospholipid could be obtained in this manner had been previously demonstrated by Perlman et al. (1938). The phospholipid was made into an emulsion suitable for injection by grinding it in a mortar with saline solution. The weight of the phospholipid was known and from this was calculated the amount of phosphorus present by assuming a P content of 3.8 per cent.

A total of 37 pregnant rats was used in this study, and from these 321 fetuses were obtained and analyzed. The gestation age was known for some, the age of others was determined by using Stotsenburg's data (1915) with comparison to fetuses of known age.

The inorganic P^{32} was supplied at different intervals and the radioactivity varied from sample to sample. The number of animals that were injected with material from any one sample was dependent somewhat on the amount of radioactive material in the sample, and hence the number of animals injected at any one time varied with the different samples. From tables 1 and 2 it is possible to calculate the activity with which each animal was injected. Each animal received 1.6 cc. of material injected into the tail vein. Each received essentially the same amount of phosphorus, whether as inorganic or as phospholipid.

Twelve pregnant rats were injected with tagged phospholipid giving a known number of counts per minute. Ten others were injected with an inorganic phosphate solution of known radioactivity. After a lapse of 2 hours the mothers were sacrificed, and the fetuses removed from the uterus in their membranes and separated from these by an electrocautery. This prevented contamination of the samples by maternal blood and also prevented any loss of fetal blood. All but one of the fetuses from each animal were pooled before being analyzed; one fetus was preserved for age determination. These pooled fetuses were

immediately macerated by grinding with ground glass, extracted with ether-alcohol, and the phospholipid isolated following the method of Bloor (1929). The phospholipid was transferred to a watch glass and the radioactivity of the sample was determined with the use of a Geiger-Müller counter of the Department of Chemistry, and corrections made for background and decay.

RESULTS. The basic data from these two groups, one injected with inorganic P^{32} and the other with tagged phospholipid, are presented in table 1. Com-

TABLE 1

Basic data on rats injected with inorganic P^{32} (upper) and tagged phospholipid (lower)

Fetuses analyzed 2 hours after injection. "Activity" refers to counts per minute as determined by the Geiger-Müller counter.

GEST. AGE	ADULT WT.	FETAL WT.	FETUSES ANALYZED	PHOSPHOLIPID RECOV'D.	ACTIVITY INJ./GM. ADULT	ACTIVITY RECOV./GM. LIPID	RATIO R/I
Injection of inorganic P^{32} into pregnant rats							
<i>days</i>	<i>grams</i>	<i>grams</i>		<i>gram</i>			
15	342	1.25	9	0.0161	871	1201	1.38
16	241	3.45	7	0.0326	186	273	1.47
16	312	4.55	10	0.0873	954	1034	1.09
17	449	2.85	4	0.0496	474	728	1.54
17	323	8.30	10	0.1077	217	302	1.39
17	354	5.80	8	0.0499	148	268	1.81
18	348	8.48	8	0.0974	361	489	1.35
18	365	9.45	9	0.1164	311	376	1.21
21	215	11.30	3	0.1954	968	876	0.91
22	332	25.15	8	0.4029	1101	1169	1.06
Injection of tagged phospholipid into pregnant rats							
16	263	2.60	8	0.0487	310	20	0.064
16	309	3.00	9	0.0630	1632	50	0.031
18	288	13.90	13	0.2863	127	36	0.281
18	261	7.88	7	0.1266	139	77	0.547
18	260	9.70	9	0.1467	140	9	0.065
18	271	8.85	8	0.1054	135	193	0.146
19	313	18.80	11	0.3316	2867	180	0.063
19	263	13.35	7	0.2052	3075	134	0.044
20	370	17.45	11	0.2259	986	100	0.101
21	264	23.10	8	0.3740	3052	131	0.043
22	321	38.75	9	0.5715	2795	748	0.267
22	348	40.50	10	0.7995	2573	192	0.075

parison of the two groups was made from the ratios of the activity recovered per gram of fetal phospholipid to the activity injected per gram of adult. Fetal phospholipid had a much higher ratio in all instances when inorganic P^{32} was injected into the mothers than when tagged phospholipid was injected. The data have been analyzed statistically and the means with the standard errors for the two groups are as follows: after injection of P^{32} , 1.32 ± 0.08 ; after injection of tagged phospholipid, 0.147 ± 0.045 ; and the difference between the means,

1.173 \pm 0.092. Each group included analyses of fetuses of different gestation ages ranging from 15 days until term. The data indicate no definite trend from which conclusions could be made on phospholipid transfer or metabolism at these different ages, although a larger sample may do so.

A third group of 15 animals was taken and each injected with inorganic P^{32} . Four of these were sacrificed at the end of 1 hour, five at the end of 3 hours, and five at the end of 6 hours and the fetal phospholipid was extracted and isolated (table 2). One animal delivered normally 5 hours after injection; the results on this case are higher than on those allowed to go 6 hours and may possibly be due to maternal blood contamination of the sample. These data

TABLE 2

Basic data on rats injected with inorganic P^{32}

Fetal phospholipid extracted at different time intervals after injection of the pregnant mothers. Further explanation in table 1 and text.

GEST. AGE	ADULT WT.	FETAL WT.	FETUSES ANALYZED	PHOSPHO-LIPID RECOV'D.	ACTIVITY INJ. GM. ADULT	ACTIVITY RECOV'D GM. LIPID	RATIO R/I	HOURS AFTER INJECT.
<i>days</i>	<i>grams</i>	<i>grams</i>		<i>gram</i>				
17	284	6.75	11	0.1530	734	252	0.34	1
19	343	22.45	13	0.3959	1067	590	0.55	1
19	302	15.15	9	0.1893	705	1253	1.78	1
21	350	35.60	11	0.5361	987	551	0.56	1
17	332	6.65	9	0.1291	1012	2378	2.35	3
17	345	5.00	9	0.0828	612	1833	3.01	3
19	292	17.60	10	0.2359	728	1913	2.63	3
19	355	19.20	10	0.3140	1001	2063	2.05	3
20	301	7.80	7	0.2735	708	2654	3.75	3
19	350	8.15	5	0.1445	610	3907	6.41	5
15	627	2.13	7	0.0603	551	1782	3.23	6
20	246	9.10	5	0.1636	859	3590	4.18	6
20	332	15.40	9	0.2303	562	3234	5.75	6
20	386	22.90	12	0.3777	922	3833	4.16	6
21	329	30.70	8	0.5213	1110	2980	2.68	6

indicate that relatively much more tagged phospholipid can be synthesized by the fetus in 1 hour following injection into the maternal circulation of inorganic P^{32} than can be formed in 2 hours if tagged phospholipid is injected. These figures also show a constant increase in the removal of P^{32} from the fetal blood and synthesis into tagged phospholipid over the time studied.

Percentage phospholipid was quite constant over the period studied, ranging generally from 1.5 per cent to 1.7 per cent of the total fetal weight.

DISCUSSION. Impressively greater synthesis of P^{32} into fetal phospholipid following injection of inorganic P^{32} into the mother as compared with the presence of much smaller amounts of the tagged element in the fetal phospholipid after tagged phospholipid is injected into the mother appears to justify the

conclusion that the placenta of the rat is relatively impermeable to the phospholipid molecule in the last 8 days of gestation. As pointed out by Needham (1931) if the lipid molecule can pass the placenta even very slowly its passage may be difficult to demonstrate by the usual methods, but the placenta may, even so, be permeable to sufficient quantities to supply the fetal needs. The present study does not settle the point as to whether the placenta is wholly impermeable to phospholipid as such, but it does indicate that the transfer of the lipid is extremely slow.

The appearance of the P^{32} in fetal phospholipid following injection of tagged phospholipid into the mother may be explained on three bases: 1, that there was some placental transfer of the intact molecule; 2, that there was a breakdown of the lipid and transfer of a smaller molecule and resynthesis on the fetal side; 3, that both processes occurred. The presence of P^{32} in the fetal lipid after injection of inorganic P^{32} into the mother might possibly be explained on the basis of synthesis into phospholipid by the maternal organism and transfer as such across the membrane. This is probably not the manner in which most of the P^{32} in the fetal lipid arrived there. Rather it is more likely that most of the P^{32} passed the placental membrane in a smaller molecular form and was synthesized by the fetus into phospholipid. That relatively much more tagged phospholipid can be synthesized by the fetus in 1 hour following an injection of inorganic P^{32} than is present in 2 hours after an injection of tagged phospholipid lends weight to the theory that most of the P^{32} gets to the embryo in the inorganic form. The lack of any definite trends in the phospholipid metabolism of the fetus in the last 8 days of gestation may well not be significant but due in the main to the small number of animals studied.

Haven and Bale (1939) have shown that in 2.5 hours after injection of radioactive phospholipid as much as 60 per cent of the injected activity may be recovered from the phospholipid of the liver and spleen. Just how much of this is immobilized by being actively phagocytosed in these organs is not known, nor are data immediately available on the amount of tagged phospholipid remaining in the blood stream at various intervals after injection. That phagocytosis is a complicating phenomenon in the present studies is recognized. However, it is interesting to note the work of Dols, Jansen, Sizoo and Barendregt (1938) on the results found after injecting inorganic P^{32} into the blood stream of rats. These workers showed that within one-half hour the injected P^{32} had entirely disappeared from the blood in some cases; at most they found but 16 per cent of the injected P^{32} still present in the whole blood. On the basis of the work of these two groups of investigators it is not likely that the differences found in the present work can be explained on the basis of phagocytosis of the injected phospholipid before it became available to the fetus.

CONCLUSION

With the aid of radioactive phosphorus as an indicator the tagged phospholipid content of the fetus has been studied in the last 8 days of gestation. On the basis of differences in P^{32} content in the fetal phospholipid following injection

of inorganic P^{32} or tagged phospholipid into pregnant rats it appears that placental transfer of the phospholipid molecule as such is a very slow process.

I am indebted to Drs. H. W. Mossman, W. van Horne and R. A. Groat for suggestions and help with the methods involved, and especially to Mr. T. P. Kohman for the preparation of most of the P^{32} and for much critical advice on technical methods and analysis of the data.

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THE ACTION POTENTIALS OF SKELETAL MUSCLES OF THE FROG

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It has been shown in preceding publications from this laboratory (1) (2) that in the frog's gastrocnemius muscle, stimulated through its motor nerve, the electrical state which gives rise to the action potential is of polar nature, i.e., consists in the simultaneous appearance of regions at which the potential is positive and of other regions at which the potential is negative, with respect to the potential of resting muscle. Because of instrumental limitations at the time, these experiments were not sufficiently refined to reveal certain important characteristics of the potential distribution such as the total number and location, at any one time, of the regions of positive and negative potentials and the possible motion of potential maxima along the long axis of the muscle. The substitution of cathode ray oscillographs and high gain, direct current amplifiers for string galvanometers, has made it possible to obtain this more detailed information. The work has also been extended to include observations on other skeletal muscles of the frog, including the sartorius, semimembranosus and biceps.

METHODS. The excised muscle, with its motor nerve, is fastened by its two ends to a fiber block in such a manner that contraction, induced by stimulation through its motor nerve, is nearly isometric. The block is placed at the center of a circular dish and the dish filled with Ringer's solution until the muscle is about half immersed. In experiments concerned with details of the potential distribution on the muscle surface, the muscle is mounted on a multielectrode block described in a previous communication (2); otherwise a plain block is used and potentials recorded by means of wick electrodes placed in contact with the muscle surface.

Two types of potential-time curves, unipolar and differential, are recorded singly or together depending upon the type of experiment. The unipolar potential-time curve is derived from a single electrode in contact with the muscle surface and a second electrode placed at the margin of the field on a line which is at right angles to the long axis of the muscle at its middle. Under these circumstances, the potential of the marginal electrode is affected to a negligible degree by potential changes at the muscle surface and the potential-time curve obtained is a record of the potential changes, with time, of the muscle surface under the electrode on the muscle. The reference potential taken in all cases is that of uninjured resting muscle.

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The differential potential-time curve is derived from two electrode contacts close together on the muscle surface. Two zinc-zinc sulphate electrodes, provided with a common wick and mounted in a holder, are employed, the wick being kept in the form of a V by a narrow celluloid strip attached to the electrode mounting and bent to exert tension on the wick. The apex of the V is applied to the muscle surface. The celluloid strip aids in preventing movement of the electrode on the muscle when it shortens. In experiments in which unipolar and differential potential-time curves are recorded simultaneously, a differential electrode is used with the addition of a single wick electrode placed at the margin of the field, as described above. Leads from one of the pair of electrodes of the differential electrode and from the marginal electrode, serve for recording the unipolar curve along with the differential curve.

The recording of the potential-time curves is carried out by means of two direct current amplifiers and cathode ray oscillographs and a camera supplied with photographic film. The amplifiers are of high gain and have an amplitude-frequency response flat within one decibel from 0 to 14,000 cycles per second. The oscillograph trace has sufficient photographic intensity to allow single exposure recording at a speed adequate for accurate measurements of the curves. The speed of recording is such that 1 mm. horizontal distance on the record corresponds to time intervals of 1 or 2 msec., the exact relation being adjusted to the experimental requirement. Records are made on stationary film, the time axes being supplied by linear electrical sweeps. The sweep voltages for both cathode ray tubes are supplied in synchronism with a condenser discharge type of stimulating current from a single instrument. The stimulating current may be varied as to duration, amplitude and as to position in the sweep. The two sweeps may also be synchronized so that events on two curves recorded simultaneously may be related with respect to time. These relations are accurately determined from the recorded curves by the use of a magnifying comparator provided with micrometers.

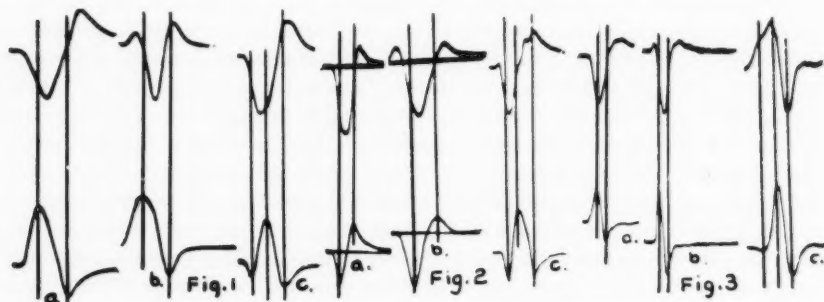
The relations between unipolar and differential potential-time curves. These two curves were recorded together from the gastrocnemius and semimembranosus muscles of the green frog, and from the gastrocnemius, sartorius and biceps muscles of the bull frog. Examples are given in figures 1, 2 and 3. The upper curves are the unipolar, the lower curves the differential potential-time records. Three examples (*a*, *b* and *c*) are given for each muscle, representing the most usual type of relations that occur between the two curves. Vertical lines are drawn through the peaks of the differential curves to designate coincident time points on the two curves. Figures 1 and 2 are from the gastrocnemius and semimembranosus muscles respectively of the green frog, and figure 3 is from the sartorius muscle of the bull frog.

Detailed examination of these curves brings out the following characteristics:

1. The general form of the two curves and their relations to each other are fundamentally the same for the different muscles studied.
2. Both the unipolar and differential curves, derived from any surface region of the muscle, are either diphasic or triphasic. In the case of the unipolar curve, this indicates that the

region under the electrode changes its electrical polarity, with respect to the potential of resting muscle, two or three times during the action potential period. The first phase may be positive or negative with respect to this reference. The potential at the region then reverses in sign and gradually returns to the potential level of resting muscle, to form a diphasic curve, or shows a second reversal to form a triphasic curve.

3. In most instances the peaks of the two curves are not coincident, but the peaks of the differential curve fall within the periods of the unipolar curve which indicate that the potential of the region is changing. Thus in figure 1a, the first peak of the differential curve falls during the period in which the initial positive potential of the region is growing (as indicated by the downstroke on the unipolar curve) and the second peak of the differential curve occurs at the



Figs. 1, 2 and 3. Unipolar (upper curves) and differential (lower curves) potential-time curves recorded simultaneously from the gastrocnemius (fig. 1) and semimembranosus (fig. 2) muscles of the green frog and from the sartorius muscle of the bull frog (fig. 3). Vertical lines are drawn through the peaks of the differential curves. For discussion, see text.

time when the potential of the region is changing rapidly from a positive to a negative value.

4. All periods during which the potential of the region is changing, as indicated by the unipolar curve, are accompanied by peaks on the differential curve, except the first potential change when the unipolar curve is triphasic, as shown in figures 1b, 2b and 3b, and during the final period of potential change (following the final peak of the unipolar curve), regardless of whether the curve is diphasic or triphasic, as shown in all the curves of figures 1, 2 and 3.

The significance of the relations between the unipolar and differential potential-time curves, recorded simultaneously, as described above, will be considered in the discussion.

The potential distribution along the long axis of the gastrocnemius and semimembranosus muscles. For the determination of the potential distribution, a series of calibrated unipolar curves is recorded from 18 to 25 separate regions along the long axis of the muscle. The multielectrode block used in previous experiments was employed for this purpose (2). In order to determine further

the instantaneous potential distribution at any given instant during the action potential period, a constant unipolar curve from some one region on the muscle surface was recorded along with the unipolar curves from the various surface regions along the long axis of the muscle. By using a peak of the constant curve

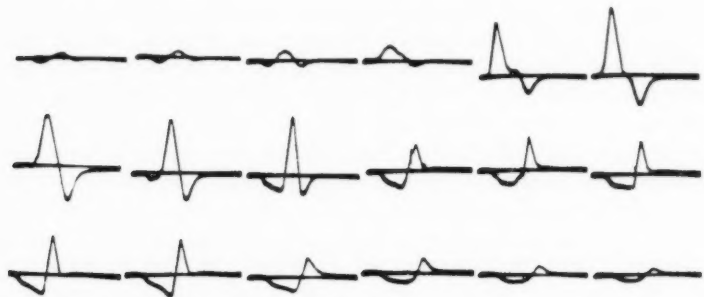


Fig. 4

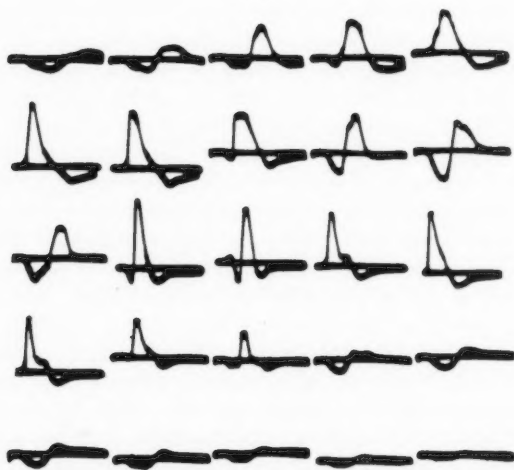


Fig. 5

Figs. 4 and 5. Unipolar potential-time curves recorded from surface points along the long axis of the gastrocnemius (fig. 4) and semimembranosus (fig. 5) muscles of the green frog, beginning at the proximal and ending at the distal ends of the muscle. The horizontal lines represent the potential of the resting muscle. For discussion, see text.

as a reference point, the potentials at the various regions for different time instants during the action potential period may be deduced in a manner described below.

In figure 4 there is given a series of unipolar curves from 18 equally spaced regions along a gastrocnemius muscle beginning at the proximal and ending at the distal end of the muscle. To conserve space, the reference curve recorded

along with each one of these curves has been omitted. The horizontal lines represent the potential level of the muscle when at rest. A movement above this line represents a negative potential, a movement below this line a positive potential, at the region of the electrode contact, and with respect to the potential of the inactive muscle. In the first three regions, passing along the muscle from the proximal toward the distal end, triphasic curves are obtained, showing that these regions are at first positive, then negative and develop a final second positive phase shortly before the action potential ends. The curve from the fourth region is diphasic; the initial positive phase has disappeared and the region is first negative and then positive with respect to resting muscle. Progressing further along the muscle, both of these potentials increase in amplitude and somewhat beyond the middle of the muscle (8th curve of the figure), an initial positive phase appears, followed by a negative and a final second positive phase, to constitute a triphasic curve as above. Succeeding this, the final positive phase disappears and the curves are again diphasic; the regions are at first positive and then negative with respect to resting muscle. This continues, along with gradual decline of both potentials, to the distal end of the muscle. In terms of polarity of the different regions along the muscle from the proximal to the distal end, we have, $+-+$, $-+$, $+-+$, $+-$.

Similar studies, carried out on the semimembranosus muscle of the green frog, show a similar polar distribution of potentials along the muscle, with certain regions showing potentials above, and others below, the potential of resting muscle, and with similar reversals of polarity. The only difference appears in a somewhat greater complexity in the semimembranosus, in the sense of a larger number of regions in which transitions through triphasic curves occur. Figure 5 shows a series of unipolar curves from 25 separate regions along the semimembranosus from the proximal to the distal end of the muscle. Beginning at the proximal end and expressing the polarities at the different regions, as above, we have, $+-$, $+-+$, $-+$, $+-+$, $+-$, $+-+$, $-+$, $+-+$, $+-$.

It is to be noted that the data as presented in the two preceding sets of records give the potential changes at the various regions of the muscle surface throughout the whole period of the action potential as a function of time. It is possible to handle the experimental records in another manner, such that the *instantaneous potential distribution along the long axis of the muscle for any given time instant is obtained*. In this case, the potential distribution is a function of the position along the muscle, time being held constant. In the first case (as presented above), there is given the potential distribution in time at any given region, while in the second case (to be described below), there is given the potential distribution along the muscle at any given time. The method of analysing the data, which will now be given, while a rather long and tedious procedure, has the important feature of giving a visualization, not only of the potential distribution, but also of the number and times of spatial movement of potential maxima (positive or negative) that may occur. In this analysis the calibrated unipolar curves from the various surface regions, each with its constant reference curve, are divided arbitrarily into some 25 to 30 equally spaced time instants and the

potential at each region for each time instant measured. The data so obtained are plotted along the long axis of the muscle for each of the time instants. Figures 6 and 7 are representative plots for the gastrocnemius and semimembranosus muscles respectively. The curves on the left of the figures are redrawings of the constant reference curve and the vertical lines drawn through these curves represent the instants during the action potential period for which the particular potential distribution along the long axis of the muscle is drawn. The time following the onset of the action potential is given in seconds to the right of each graph. The horizontal lines in each graph represent the potential of resting muscle. Plottings above this line represent negativity, those below positivity, with respect to this potential reference. Each graph thus represents the potential distribution along the muscle at the particular time instant after the beginning of the action potential period as indicated by the figure to the right. Further details as to the significance and analysis of these graphs will be given in the discussion.

DISCUSSION. A large part of the work on the action potentials of skeletal muscle that is found in the literature has been carried out on isolated curarized muscle stimulated directly. Curarization was done with the idea of preventing the stimulation of the muscle through its nerve supply. There is no question however that curare produces marked alteration in the physiological response of muscle and that its action is not confined to paralysis of the motor end plates. The normal physiological response of skeletal muscle occurs with intact nervous connections and as a result of impulses reaching it through its motor nerve. This has been recognized by some workers, notably Fulton (3), who states "we cannot expect to study the physiological behavior of electrical responses on curarized muscle. The action current of an intact muscle stimulated through its motor nerve must be the object of our study, whatever other inconveniences it involves."

Attempts at the interpretation of the electrical state existing in active skeletal muscle have been carried out, with few exceptions (4), by recording one of two types of leads, two leads from the surface of the muscle, spaced a considerable distance apart ("bipolar leads"), or one lead from an injured region on the surface of the muscle or from the tendon, the other from an uninjured surface region ("monophasic action potential"). Since in a bipolar lead a given deflection in the curve may result from a potential change of one sign under one electrode, or a potential change of the opposite sign under the other electrode, and since it is impossible to distinguish between these, the curve cannot be used for an analysis of potential distribution. The "monophasic action potential" in heart muscle has been shown to be an entirely different phenomenon than it has been previously assumed to be, and not indicative of a fall of potential at the "active" or "different" electrode on the uninjured region (5). What it represents in skeletal muscle has not been demonstrated, and interpretation of the normal action potential based on such leads, must, at the present time, be seriously questioned.

In the following discussion of the experimental results described in the preced-

ing section, it is important to have clearly in mind the significance of the unipolar and differential potential-time curves as recorded from the muscle surface and of certain relations that exist between these two curves. The unipolar potential-

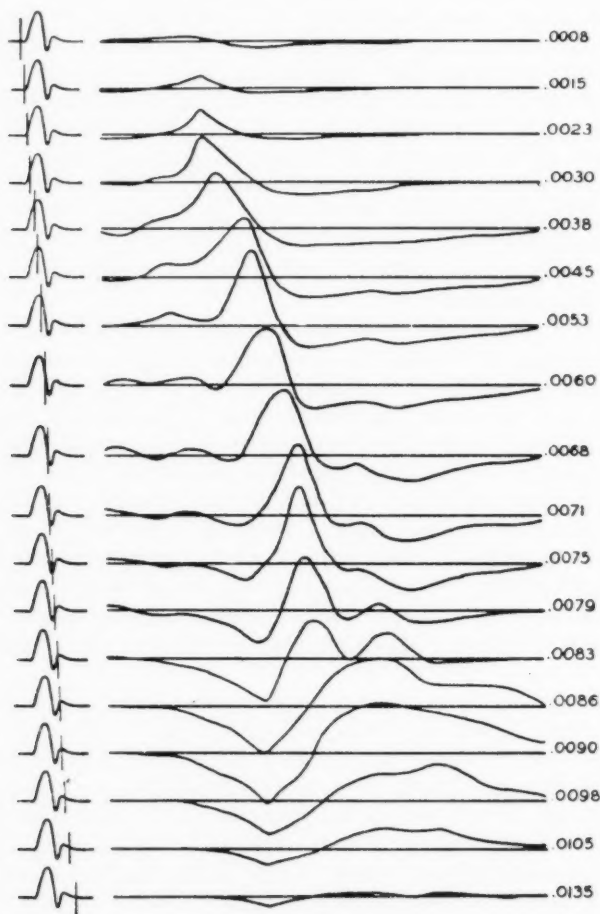


Fig. 6

Figs. 6 and 7. Instantaneous distribution of potentials along the surface of the gastrocnemius (fig. 6) and semimembranosus (fig. 7) muscles of the green frog at different times in the action potential period. The horizontal lines represent the potential of resting muscle. For discussion, see text.

time curve is a record of the potential variations of the muscle under the electrode with time and with respect to the potential of the muscle during the resting state. Peaks represent potential maxima, positive or negative, and the slopes

of gradients of the curve represent the time rate of change of potential in the region.

The differential potential-time curve, derived from two points on the muscle surface close together, is a record of the potential differences between these two points with time. For the record to be a differential potential-time curve the points must be sufficiently close together such that at any instant the potential

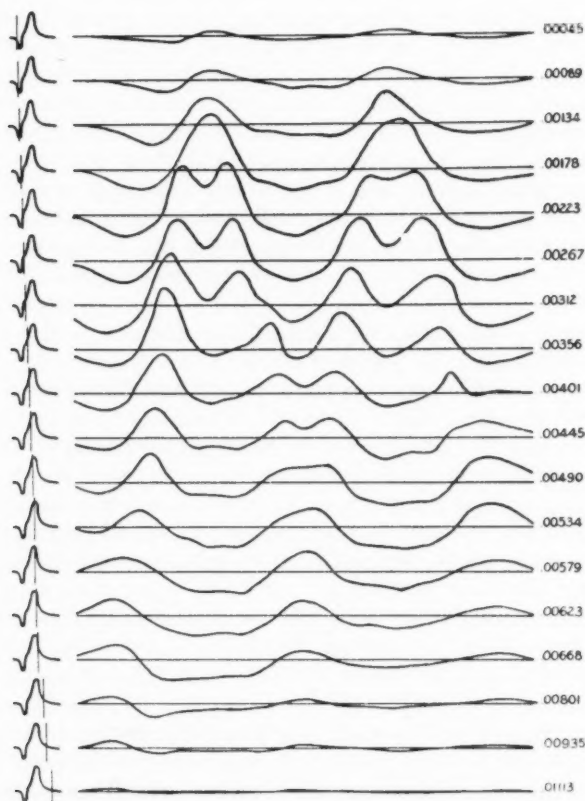


Fig. 7

variation between the two is linear or nearly so. If this condition is met, the potential difference between them at any instant is proportional to the electrical gradient existing between the two points at this instant. Since the current density is equal to the product of the electrical gradient and the electrical conductance, this potential difference, whose time variation is the differential record, is proportional to the current flow between the two points. The differential curve is therefore a measure of variations, with time, of current flow in the region between the two contacts of the electrode. Periods of maximum flow of electrical

current are coincident with peaks on the differential curve and slopes or gradients of the curve represent the time rates of change of current flow in the region.

It may be shown mathematically (see appendix for mathematical treatment) that certain time relationships between the peaks of the differential curve and the time gradients of slopes of the unipolar curve, and certain similarities or dissimilarities in the shapes of the two curves, can occur only if the change in potential distribution from one instant to the next occurs in certain specific ways. In order to discuss this matter, it is necessary to consider the ways in which the potential distribution on the surface of the muscle varies from time to time. In figures 6 and 7, any one of the set of curves is a graph of the potential, relative to that of the resting state, during one instant of the action potential along the long axis and surface of the muscle. We may speak of this graph as a potential function, since it expresses the potential at the instant as a function of the distance along the long axis of the muscle, with respect to the potential of the muscle in the resting state. Certain regions are negative, while other regions are positive. In any positive region there will be a point, represented by a peak on the unipolar curve, when the potential is maximum in the region. Likewise in a negative region, there will be a curve peak corresponding to the maximum negative potential. The potential distribution may change from time to time throughout the period of action potential in two ways. Growth or decline of the potential may take place without any shift of peaks, or it may be associated with displacement along the muscle of the potential maxima. Both types of behaviour are apparent in figures 6 and 7. In the first circumstance the potential function is nonpropagated, i.e., it shows growth or decline in amplitude but the maxima do not shift from one instant to the next. In the second circumstance, the potential function is propagated; along with growth or decline in the amplitude of the potential function, there is motion of potential maxima from one instant to the next.

Mathematical considerations show that in an electrical state characterized by a non-propagated potential function, the shape of the unipolar and differential potential-time curves will be similar and their peaks coincident. The converse is also true; similar curves and coincident peaks occur only if the potential function is non-propagated. If, however, the magnitude of the differential curve at each time instant is proportional to the slope of the unipolar curve, the potential function is propagated and shows neither growth nor decline. Under these circumstances, the differential curve is the first derivative with respect to time of the unipolar curve. The peaks on the two curves will not be coincident, but the peaks on the differential curve will occur during the periods on the unipolar curve which indicate that the potential is changing and will be of a magnitude proportional to the time rate of change of potential. If the potential function is propagated and also growing or declining, or if the velocity of propagation is not constant, the situation is more complex, and the relation described between the unipolar and differential curves is true only to a first approximation.

In previous work reported from this laboratory, unipolar and differential potential-time curves were recorded simultaneously from the surface of the

auricles and ventricles of the dog and turtle heart (6) (7). The relation between the two indicated that the potential function is a propagated one throughout the QRS period of action potential. Peaks appear on the differential curve coincidentally with each gradient on the unipolar curve and the amplitudes of the peaks are approximately proportional to magnitude of the gradients. That the potential function in the heart is a propagated one was also shown by mapping the potential field on the surface of the ventricle of the turtle.

The relationship between differential and unipolar potential-time curves from skeletal muscle, as described in the present communication, does not fall wholly in one category or the other and would indicate that in this case we are dealing with a potential function which is in part non-propagated and in part propagated. At the very beginning of the action potential period and during approximately the last half, the relationship predicts a non-propagated potential function, while the first half, with the exception of the beginning, it predicts the existence of a propagated potential function during this period. These predictions, based on the relationship between the unipolar and differential potential-time curves, are amply verified by mapping the potential field along the surface of the muscle, as will now be shown.

The potential distribution at different time instants during the action potential period along the long axis of the gastrocnemius muscle (fig. 6) may be described as follows: The potential distribution is polar, i.e., regions of positive and negative potential exist simultaneously. The first three milliseconds of the action potential period are characterized by the development, without movement, of a region of negative potential near the proximal end and a region of positive potential near the middle of the muscle. The potentials in both regions grow in magnitude and after the third millisecond show movement of the potential maxima toward the distal end of the muscle, the movement continuing up to about the seventh millisecond. At this time reversal of polarity begins, a region of positive potential developing toward the proximal end and a region of negative potential developing toward the distal end of the muscle. These grow and decline with little or no motion. In terms of polarity, with respect to time in the action potential period, the potential distribution from the proximal to the distal end of the muscle is expressed as, $- +, + -$. This distribution of positive and negative potentials is the same, except for added details as to movement of the potential maxima as that which was derived from previous studies concerned with the potential distribution in a conducting field surrounding the gastrocnemius muscle.

While the gastrocnemius muscle, which is relatively complex anatomically, gives a fairly simple picture of potential distribution, the semimembranosus muscle, which is described as a long straight fibered muscle (8), gives a picture more complex in detail but fundamentally similar in its broader aspects (fig. 7). The potential distribution in the semimembranosus is more complex in the sense of a larger number of regions of positive and negative potentials and in the movements of the potential maxima during a part of the action potential period. During the first 2 msec. regions of positive and negative potentials appear, with

the following arrangement, starting at the proximal and proceeding to the distal end of the muscle; $+-$, $-+$, $+-$, $-+$. These increase in amplitude and show little or no motion of potential maxima. From the 3rd to the 6th msec., rather complicated movements of the potential maxima occur. The outer pairs of potential maxima move toward the two ends, while the inner pairs move toward the middle of the muscle. Reversals of polarities appear toward the end of this period, giving rise at the 6th msec. instant to the following arrangement; $-+$, $+-$, $-+$, $+-$. These grow and decline with little or no motion up to the end of the action potential period.

The movement or non-movement of potential maxima during certain parts of the action potential period, predicted from the relation between the unipolar and differential potential-time curves, has thus been verified in the two muscles in which the potential distribution along the muscle has been determined. There would seem to be ample justification for the conclusion that a similar type of movement of potential maxima occurs during the action potential period in the sartorius and biceps femoris muscles, and that this feature is a characteristic one for the skeletal muscle of the frog. The relation between the unipolar and differential potential time curves is the same in all four of the muscles studied, and our results would indicate that this relationship alone is sufficient to reveal this characteristic.

It is evident from the present and preceding work that the potential distribution which gives rise to the action potentials from the heart during its normal activity and from skeletal muscle when stimulated through its motor nerve, are fundamentally similar in that they both have a polar distribution, regions of potentials, positive and negative respectively, with reference to the potential of the inactive muscle, arising simultaneously, undergoing growth and decline, movement and reversal of polarity. Movement of potential maxima occurs throughout the whole of the QRS period of the action potential period in the heart, while this motion is restricted to the mid period of the action potential of skeletal muscle. While movements of potential maxima occur during a part of the action potential period of skeletal muscle when activity is brought about, it is to be noted that there is no evidence to indicate a progressive wave of electrical involvement from one end of the muscle to the other, such as has been described in curarized skeletal muscle stimulated directly.

It is quite possible that the finer details of the differences in potential distribution in different skeletal muscles may depend on the innervation and the pattern of activation of different muscle groups. It is also possible that potentials developing in terminal nerve fibers or in end plates may contribute to the total action potential of the muscle. Our present state of knowledge is not sufficient to permit an adequate discussion of this aspect of the problem. The matter of fundamental interest at present appears in the fact of similarities of potential distribution rather than in details of their differences in different muscles.

SUMMARY

The potential distribution on the surface of the skeletal muscle of the frog, during contraction brought about by a single stimulus to its motor nerve, differs

from that on the surface of the normally contracting heart, in that movement of potential maxima occurs during only a part of the action potential period. This difference was predicted on theoretical grounds because of the observed difference in the relationship, in the two cases, between the unipolar and differential potential-time curves recorded simultaneously from the same surface region, and experimentally validated by mapping the potential fields on the gastrocnemius and semimembranosus muscles. In the fundamental aspect of the polar nature of the potential distribution, the heart and the skeletal muscle are the same; regions of positive and negative potentials, with respect to the potential of resting muscle, developing coincidentally, undergoing growth and decline, certain displacements and reversals of polarities.

Mathematical appendix. Let $P(x, y, z, t)$ represent the potential distribution in the conducting field for all points x, y, z and all times, t . Any unipolar lead derived from a point in the field x_0, y_0, z_0 , and a contact at the edge of the field will give a time distribution record

$$P(x_0, y_0, z_0, t)$$

A differential lead derived from x_0, y_0, z_0 and a point close to it, $x_0 + \Delta x, y_0 + \Delta y, z_0 + \Delta z$ will give a time distribution curve represented by $N(x_0, y_0, z_0, t)$.

By Kirehoff's law

$$N(x_0, y_0, z_0, t) = P(x_0 + \Delta x, y_0 + \Delta y, z_0 + \Delta z, t) - P(x_0, y_0, z_0, t)$$

Thus, at any point x, y, z , the unipolar time distribution is $P(x, y, z, t)$; the associated differential time distribution is $N(x, y, z, t)$ and

$$N(x, y, z, t) = P(x + \Delta x, y + \Delta y, z + \Delta z, t) - P(x, y, z, t)$$

If x, y, z are of differential dimensions, i.e., so that the variation of $P(x, y, z, t)$ is linear in the region, $\Delta x, \Delta y, \Delta z$ may be replaced by dx, dy , and dz .

If this is true, it may be also said that $N(x, y, z, t)$ is equal to differential $P(x, y, z, t)$ written as

$$N(x, y, z, t) = dP(x, y, z, t) \quad \text{or}$$

$$N(x, y, z, t) = \frac{\partial P}{\partial x} dx + \frac{\partial P}{\partial y} dy + \frac{\partial P}{\partial z} dz$$

Let us agree in placing the differential lead contacts to place them always so that a line through the two contact points always makes the same angles with the x, y and z axes; this direction to be arbitrarily chosen and held fixed throughout the discussion. Let us call the direction cosines of this line with the x, y and z axes c_x, c_y and c_z respectively. Let us also agree to keep the separation of the two contacts constant and equal to a value, L , this value, L , being sufficiently small as required. Then

$$N(x, y, z, t) = L \left(c_x \frac{\partial P}{\partial x} + c_y \frac{\partial P}{\partial y} + c_z \frac{\partial P}{\partial z} \right)$$

Case 1. Time distribution curve of differential lead proportional to time derivative of time distribution curve of unipolar lead.

This may be represented as

$$N(x, y, z, t) = K \frac{\partial}{\partial t} P(x, y, z, t)$$

where K is a constant of proportionality. Therefore

$$c_x \frac{\partial P}{\partial x} + c_y \frac{\partial P}{\partial y} + c_z \frac{\partial P}{\partial z} = K/L \frac{\partial}{\partial t} P(x, y, z, t)$$

A general solution of this differential equation is

$$P(x, y, z, t) = \varphi \left(\frac{\alpha x}{c_x} + \frac{\beta y}{c_y} + \frac{\gamma z}{c_z} + \frac{L\delta}{K} t \right)$$

where

$$\alpha + \beta + \gamma = \delta$$

This is a purely-propagated potential function and is a plane wave propagated with a velocity

$$\frac{L\delta}{K} \sqrt{\frac{\alpha^2}{c_x^2} + \frac{\beta^2}{c_y^2} + \frac{\gamma^2}{c_z^2}}$$

along a line whose direction cosines are

$$c_x \sqrt{\frac{\alpha^2}{c_x^2} + \frac{\beta^2}{c_y^2} + \frac{\gamma^2}{c_z^2}}, \quad c_y \sqrt{\frac{\alpha^2}{c_x^2} + \frac{\beta^2}{c_y^2} + \frac{\gamma^2}{c_z^2}}, \quad c_z \sqrt{\frac{\alpha^2}{c_x^2} + \frac{\beta^2}{c_y^2} + \frac{\gamma^2}{c_z^2}}$$

This means that the existence of the situation defined as case 1 implies the presence of a purely propagated potential function.

Case 2. Time distribution curve of differential lead proportional to time distribution curve of unipolar lead. The constant of proportionality may vary from point to point. This may be represented by

$$N(x, y, z, t) = f(x, y, z)P(x, y, z, t)$$

where $f(x, y, z)$ represents the constant of proportionality at any point x, y, z . This results in the differential equation

$$L \left(c_x \frac{\partial P}{\partial x} + c_y \frac{\partial P}{\partial y} + c_z \frac{\partial P}{\partial z} \right) = f(x, y, z)P(x, y, z, t)$$

A general solution of this equation for $P(x, y, z, t)$ is

$$P(x, y, z, t) = V(x, y, z)T(t)$$

where $V(x, y, z)$ is a solution of the differential equation

$$L \left(c_x \frac{\partial V}{\partial x} + c_y \frac{\partial V}{\partial y} + c_z \frac{\partial V}{\partial z} \right) = f(x, y, z)V(x, y, z, t)$$

The solution for $P(x, y, z, t)$ takes the form of a non-propagated function in this case. Therefore, the existence of case 2 implies the existence of a non-propagated potential distribution.

It should not be expected that the results of any actual experiment will fit wholly into either case 1 or 2 or that the fit in any case will be any better than approximate. The analysis is exact with respect to the conditions stipulated but should apply only approximately to experimental situations which approximate the conditions. Its value is dependent on the experimental verification of predictions based on it. Fortunately, the instantaneous field determination method may be used as a check.

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CORTICAL RESPONSES TO ELECTRIC STIMULATION

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The original purpose of this study was the elucidation of some of the interesting problems offered by the continuing after-effects of electrical stimulation of the motor cortex (experimental epilepsy). Thus, the mechanism of conduction which determines the spread of these effects, and the factors which control the rate of their occurrence were questions which challenged explanation. As the study developed and experiments suggested new questions that purpose was enlarged to include several different types of cortical responses which can be elicited by stimulation of any cortical area.

Among the previous publications dealing with some of the topics with which this paper is concerned the following may be mentioned: Bubnoff and Heidenhain (1881); François-Francé and Pitres (1883); Adrian (1936); Bishop and O'Leary (1936); Bremer (1938); Dusser de Barenne and McCulloch (1938, 1939); Moruzzi (1939), and Erickson (1940). The present effort attempts to

enlarge upon these previous contributions and also to emphasize some features common to various cortical responses.

METHODS. The animals used were mainly rhesus monkeys, under chloralose anesthesia (0.06 to 0.08 gram per kgm.). This choice was determined by the observation of Rioch and Rosenblueth (1935) that stimulation of the cortex of monkeys under that anesthetic readily results in prolonged (several minutes) marked motor after-effects. Occasional observations for purposes of comparison were made on dogs, anesthetized also with chloralose, and on cats, under nembutal anesthesia.

One or both cerebral hemispheres were largely exposed. Small light brass rods were screwed into suitable regions of the remaining cranial bones. These rods supported firmly the stimulating and recording electrodes. Thus movements of the animal did not cause shifts of contact between the electrodes and the brain.

For the study of motor responses one or two muscles were attached to myographs. The muscles were gracilis, semimembranosus, quadriceps, or flexor sublimis digitorum. When recording from the leg muscles the femur was fixed by means of drills; the tendons were attached to tension myographs and the contractions were recorded on a kymograph. When flexor digitorum was used the fore-arm was fixed by means of heavy steel needles inserted into the bones and held by clamps; the tendons were attached via pulleys to a torsion-spring myograph of the Sherrington type. The beam of light from the myograph was reflected to the back of the film used for simultaneous photographing of cortical electric responses from the cathode-ray oscillograph. Muscular electric responses were led to the amplifier by two silver needles or by concentric electrodes of the Adrian-Bronk type.

The electric phenomena in the cerebral cortex were recorded either from 2 electrodes on the surface or from one applied to the surface and another inserted to variable depths, usually about 3 mm. This last electrode was a fine silver wire, insulated except at the tip. The surface electrodes were likewise silver wires, with a small bead at the tip to insure a good contact without damage to the nervous tissue; they were applied above the pia.

Capacity-coupled amplification of the electric responses was used routinely, and only occasional observations were made with a direct-coupled amplifier. The time constant of the capacity-coupled amplifiers could be varied from 0.05 sec., for the observation of rapid phenomena, to 0.5 sec., for the study of slower events. The amplified signals were led either to 1 to 6 ink-writing galvanometers and recorded on moving paper, or they were led to 1 to 3 cathode-ray oscillographs and photographed. The usual procedure was to ground the animal by an indifferent large electrode on muscle and to lead to the amplifiers on push-pull.

The stimuli were either induction shocks from a Harvard coil or, more commonly, condenser discharges through a thyatron, delivered directly or rendered diphasic by means of a transformer. The intensity of the stimuli was carefully adjusted to avoid spread of the currents to adjacent regions

when such spread could vitiate the interpretation of the results. The stimulating electrodes were similar to those used for "surface" recording. The interelectrode distances varied from 3 to 8 mm.

RESULTS. A. *Motor Responses.* a. *Different types of motor responses.* Stimulation of the motor cortex (area 4) with weak shocks or with slow frequencies results, after a relatively brief latency, in contraction localized to certain muscles. This contraction builds up rapidly and does not outlast significantly the period of stimulation. This first type of response may be spoken of as "direct," since it is probably due to direct activation of pyramidal projection elements (Dusser de Barenne, 1934). The cortical region from which a given muscle may be stimulated directly will be referred to as the "primary motor region," or "point," for that muscle.

Relatively weak stimulation of regions in area 4 other than the primary motor region for a recording muscle may lead, if sufficiently prolonged, to the gradual building up of a contraction. This response again promptly subsides when the stimuli are stopped (cf. fig. 2). This second type of response will be referred to as "indirect and unsustained." The term "indirect" emphasizes that the cortical projection elements to the recording muscle are not activated directly by the stimuli, but indirectly, through other cortical neurons. The adjective "unsustained" stresses the difference between these responses and the third type.

Intensification or increase of the frequency of the stimuli or of the duration of the period of stimulation of a primary motor region or of neighboring areas results in the appearance of the well-known tonic-clonic sequence of motor effects (experimental epilepsy). This activity, as opposed to the direct effects, bears little correlation to the stimuli which evoke it. Its rhythm is largely independent of that of the stimuli. Instead of remaining localized it tends to spread. It differs from the unsustained effect in that it long (up to 5 min.) outlasts the period of stimulation. The continuing after-effects indicate self-sustained activity. Cortical efferent elements may be activated indirectly, since muscles not directly connected with the stimulated region readily participate. The tonic-clonic motor sequence may therefore be referred to as an "indirect, self-sustained response."

b. *The tonic-clonic sequence.* If the primary motor region of a recording muscle is sufficiently stimulated the direct effects are immediately followed by the indirect activity (fig. 1A). When other areas than the primary motor area are stimulated unsustained effects may develop, followed later by the tonic-clonic effect (fig. 2). Instances of pure self-sustained responses are illustrated in figure 3.

As shown in figures 1, 2, and 3, the tonic-clonic sequence shows typically an initial period of sustained high tension. This is followed by comparative inactivity. Then there appear fast and irregular phasic movements. The relatively slow clonic bursts end the response. The complete sequence should therefore be described as tonic-depressed-phasic-clonic. For brevity the designation "tonic-clonic" has been adopted. Many atypical records were seen.

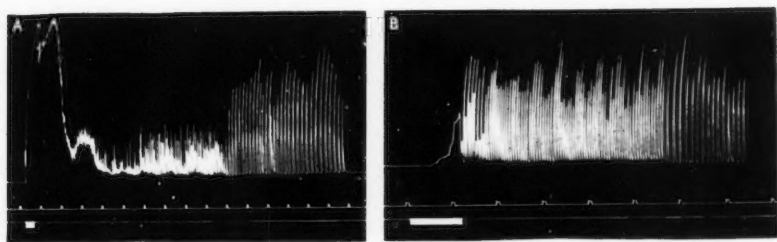


Fig. 1A. Direct muscular response to stimulation of the cortex, followed by typical tonic-clonic after-effects. Monkey. Record of left gracilis muscle. The lower signal indicates stimulation of the right motor leg area with induction shocks of tetanic frequency (coil distance; 6.5 cm.). In this and the following kymograph records the time signal corresponds to 5-sec. intervals.

B. Sudden change of rate of clonic contractions. Monkey, left gracilis. Stimulation of lower part of right motor arm area—i.e., near face area.

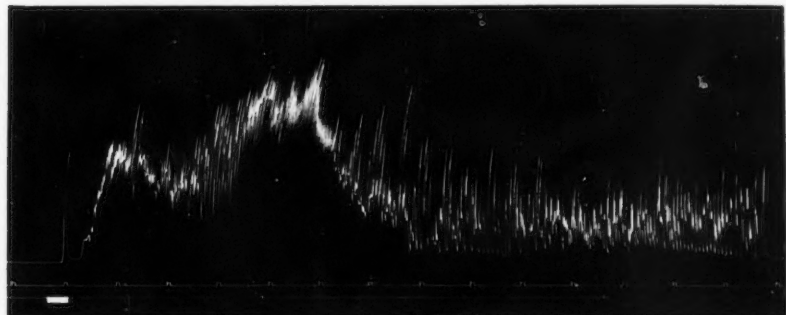


Fig. 2. Indirect unsustained response followed by tonic-clonic effects. Monkey, left gracilis. Stimulation of right motor arm area.

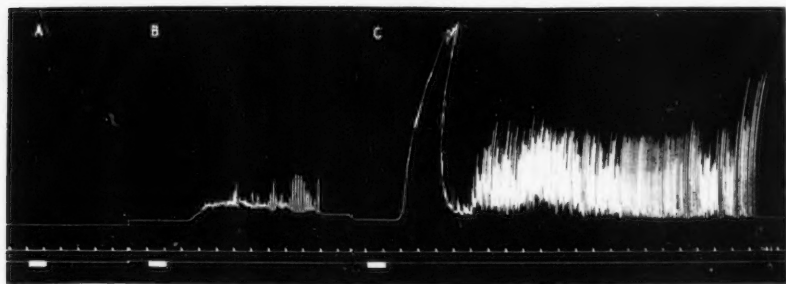


Fig. 3. Indirect (tonic-clonic) motor responses to cortical stimulation. Influence of intensity of stimuli. Monkey, left gracilis. Stimulation of right motor arm area with induction shocks of tetanizing frequency. Coil distances: A, 7.0; B, 6.5; and C, 6.0 cm. In A, although there was no motor response of the recording leg muscle, the left arm showed a typical tonic-clonic response.

One or more of the four stages mentioned could be absent (see fig. 1B). Slow clonic contractions could be interrupted by rapid phasic bursts.

The end of the response was usually abrupt. Without much slowing of the rate of clonic activity, and without any decrease in the amplitude of the contractions—indeed, with a progressive increase (fig. 3)—the response stopped suddenly. Occasionally, however, after the regular series of clonic contractions had stopped for a few seconds, there followed 2 or 3 additional contractions. Occasionally, also, the amplitude of the clonus progressively decreased till disappearance.

Usually the rate of the clonic contractions slowed gradually during the response from about 3 to 1 per sec. Not infrequently, however, the rate changed suddenly in the course of a discharge to about one-half the previous value. This sudden change of rate was not marked by any significant modification of the amplitude of the contractions (fig. 1B).

c. Influence of the characteristics of the stimuli. Weak stimulation of a primary motor point elicited only direct effects, not followed by self-sustained

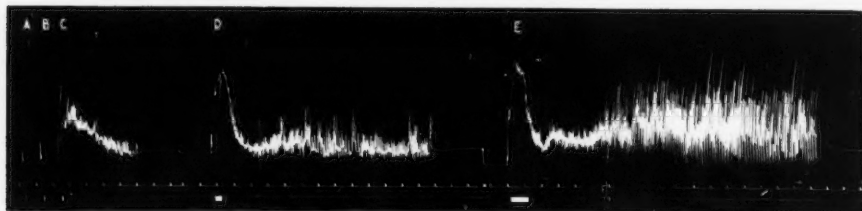


Fig. 4. Influence of period of stimulation on tonic-clonic after-effects. Monkey, left gracilis. Stimulation of right motor leg area with induction shocks (coil distance 6.0 cm.) for the following periods: A, 0.2; B, 0.5; C, 0.8; D, 2.0; and E, 5.0 sec.

activity. Weak stimulation of regions other than the primary motor point caused no contraction of the recording muscle. Intensification of the stimuli resulted in progressively greater self-sustained responses (fig. 3).

For a given intensity and duration of the stimulating shocks a critical frequency was found, both for monkeys and for dogs, below which no indirect responses could be elicited. Thus, with shocks of moderate intensity no self-sustained activity followed stimulation at rates less than about 10 per sec. With strong shocks, however, tonic-clonic responses could be produced after 10 to 15 sec. of stimulation at rates as slow as 3 per sec. Increasing rates of stimulation above the critical value (up to 120 per sec.) caused a corresponding increase of the amplitude and duration of the after-effects.

Prolonging the period of application of adequate stimuli resulted, as a rule, in greater indirect effects (fig. 4). An optimum duration, however, was encountered; long periods of stimulation resulted in reduced or absent after-effects (fig. 5).

d. Cortical areas from which indirect motor effects can be elicited. For refer-

ence to the different areas in the cortex of the rhesus monkey the diagram of Brodmann (1905) for the cercopithecus will be used, as adapted by Dusser de Barenne and McCulloch (1938). The term "motor cortex" has been employed thus far to indicate area 4.

Several other areas than 4 can be stimulated to bring forth tonic-clonic responses of a given muscle. The following general statements summarize the experimental findings. The region of the contralateral area 4, from which direct responses of a given muscle are most readily elicited—i.e., the primary motor region—is also the region from which with a given stimulus maximal self-sustained results will ensue. The greater the distance between any cortical point or area in the same hemisphere, and the primary motor point, the more difficult it is to produce tonic-clonic effects on the given muscle, or the less the effects of a given stimulus. Although motor responses are in general more easily evoked by stimulation of area 4 and also quite readily from area 6, stimu-

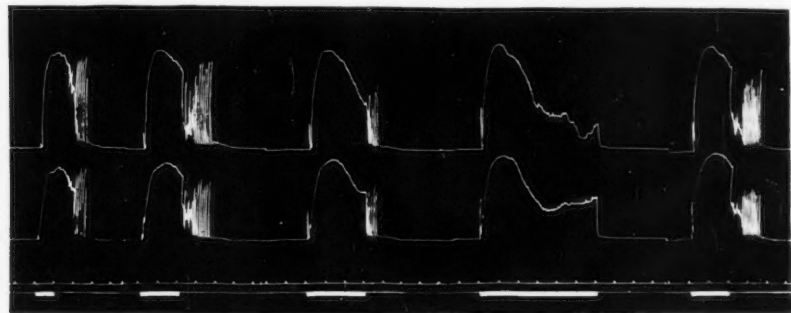


Fig. 5. As in figure 4, but showing that there is an optimum duration of the period of stimulation. Dog. Upper record: left quadriceps, and lower record: left gracilis. Stimulation of the right motor leg area with induction shocks, as shown by the lower signals (coil distance 6.0 cm.).

lation of other areas, e.g., 8, 9, 1, 2, 5 and 7, may result in well-developed tonic-clonic sequences. These responses are not due to spread of current, but to spread of activity, since the motor effects may not begin until well after the stimuli have ceased. The distance from the stimulated point to the "primary" motor point is more important for the appearance of a motor response than the area to which that stimulated point belongs.

Stimulation of the opposite hemisphere—i.e., ipsilateral to the recording muscle—, as noted above, results in tonic-clonic movements. Maximal effects are seen when the point symmetrical to the primary motor point is stimulated. The effects decrease with the distance between the area stimulated and that symmetrical point. The decrease with distance is greater than that which occurs in the hemisphere contralateral to the recording muscle—i.e., the circle around the primary motor point from which tonic-clonic responses may be elicited is larger than the circle around the symmetrical point in the opposite hemisphere.

Considerable variability was found among different animals with regard to

the spread of activity from stimulation of a given area. In some animals motor responses to ipsilateral cortical stimulation were very readily seen; in others such ipsilateral effects could not be obtained. In some animals extensive spread took place at the contralateral hemisphere, in others the responses, even if striking and prolonged, tended to remain localized to the muscles under direct control. In general, marked spread, ipsilateral and contralateral, was best seen in animals under light anesthesia and with the cortex freshly exposed. But even in similar conditions large individual differences were common.

c. *Facilitation and inhibition.* The description has dealt thus far with the results of single periods of stimulation of a "rested" cortex. The effects of previous on shortly following stimulation are considered here.

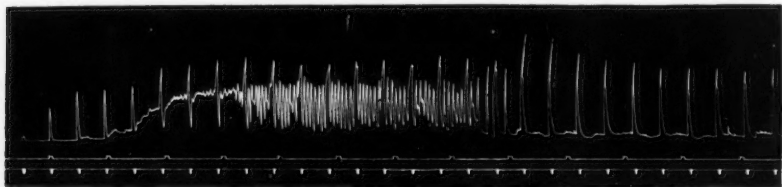


Fig. 6. Summation of the effects of brief periods of repetitive stimulation. Dog, left quadriceps. The lower signals mark the periods of stimulation of the right motor leg area with induction shocks.

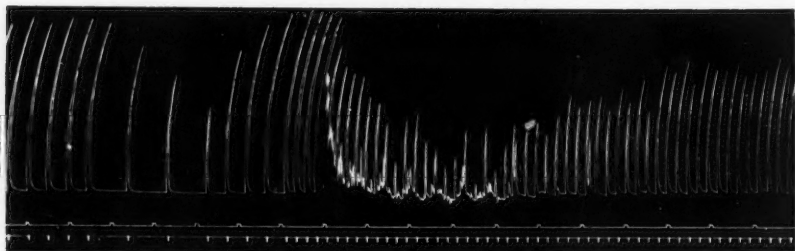


Fig. 7. As in figure 6, but record from the gracilis of a monkey. See text for further explanation.

In a series of observations a train of stimuli was selected which did not produce any indirect motor effects, and which usually gave a slight direct response. Such stimulation was then repeated regularly at intervals of 1 to 5 sec. Within those intervals a summation of subliminal indirect effects was seen regularly. First the direct responses grew; and after a few applications a typical tonic-clonic sequence developed. If the stimuli were continued the direct responses were present during and after the tonic-clonic effect. There was a difference between dogs (fig. 6) and monkeys (fig. 7) in this phenomenon. In dogs the direct responses were greater throughout the tonic-clonic self-sustained activity and for some time afterwards than they were at the start. During the tonic period the direct contraction, superimposed on the background tension, was

followed by a brief relaxation. The observations in monkeys differed from this description in two features; the direct contractions during tonus were not followed by inhibition; they were decreased or absent during the clonic period.

If the series of stimuli was prolonged after the subsidence of a first tonic-clonic response only large direct effects were recorded for some time (15 sec. to 3 min.). A second and later a third tonic-clonic reaction could then develop, similar to the first one.

Figure 7 illustrates the influence of the intervals between stimuli on the amplitude of the direct effects. The record begins after a series of brief trains of induction shocks had been applied for 90 sec. A tonic-clonic response had developed and subsided. Slowing of the series at the beginning of the record resulted in a decrease of the direct responses. Later acceleration produced an increase of these direct effects and caused the development of a second tonic-clonic response.

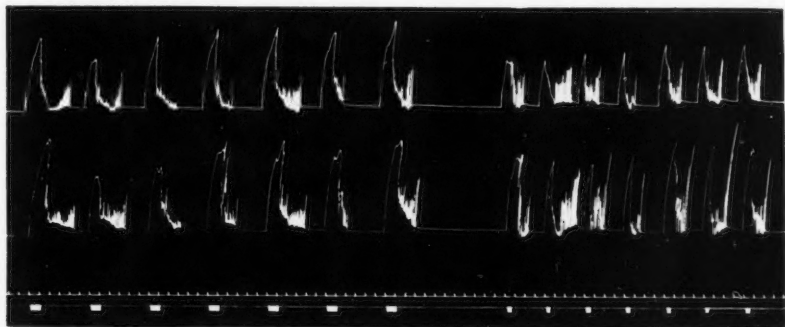


Fig. 8. Repeated tonic-clonic responses at short intervals. Dog. Upper record, left gracilis, and lower record, left quadriceps. Stimulation of the right leg motor area with induction shocks (coil distance 5.5 cm.), as shown by the signals.

If a stimulus which caused a tonic-clonic response was repeated immediately at the end of the reaction only direct, but no indirect motor effects ensued. For accurate reproduction of the original effects a rest pause of 15 sec. to 5 min. was necessary before reapplication of the stimulus. When the tonic-clonic response was brief, however, the initial effects could be approximately duplicated even with rest intervals of only 5 to 15 sec. Figure 8 illustrates two series of responses obtained at short intervals in a dog.

B. Self-Sustained Electric Responses of the Cortex. a. *The electrogram of the primary motor region during the tonic-clonic motor response.* The electric responses of the cortex were recorded either from two electrodes (2 to 8 mm. apart) placed on the surface or from a surface electrode to another, insulated except at the tip and inserted about 3 mm. directly below the one on the surface. For convenience the records obtained with the surface leads will be referred to as "surface" records or corticograms; those obtained from surface to underlying white matter will be called "transcortical" records.

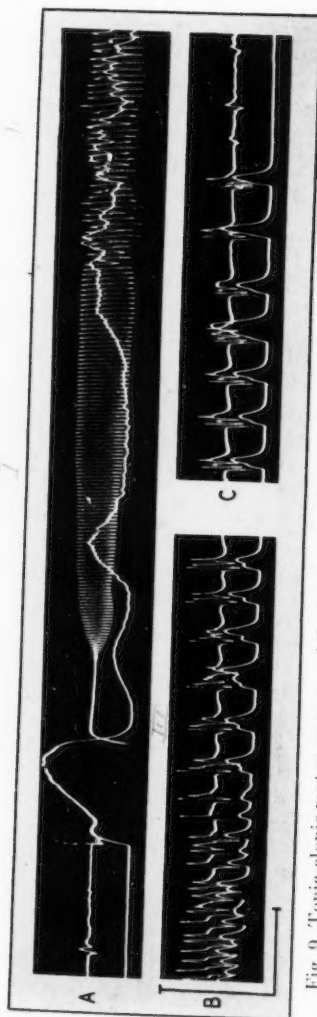


Fig. 9. Tonic-clonic motor response of the left finger-flexors (lower tracing) and cortical surface electrogram from the corresponding primary motor point in the right 4 cm area (upper tracing). The right 4 face area was stimulated with induction shocks at the time in A where the electrical record disappears, because of the large rapid signals corresponding to the stimulus artifacts. B and C are records 25 and 45 seconds, respectively, after the period of stimulation.

In this and other records of electrical responses the following conventions are adopted. The term "surface" record or electrogram implies that the recording electrodes were on the surface of the cortex, 2 to 8 mm. apart; the term "transcortical" record signifies that the recording electrodes were, one, a needle inserted 2 to 4 mm. deep and insulated except at the tip, the other, a needle on the surface directly above the tip of the inserted needle. In the transcortical records upward excursions in the tracing indicate negativity of the surface with respect to the deep cortical layers. Unless otherwise stated the records were taken in monkeys, with capacity-coupled amplifiers, and with a cathode-ray oscillograph. The two perpendicular lines at the left lower corner of the figures have the following meaning: the horizontal corresponds to 1 sec., the vertical to a potential difference which will be indicated for each figure. In this case the amplitude of the vertical corresponds to 2 mv.

Figure 9 illustrates a surface electrogram from the primary motor point of the left digital flexors, together with the mechanogram of the muscles in response to stimulation of the right motor cortex in the neighborhood of the recording electrodes. The position of the primary motor point was determined before applying the recording leads by careful, just threshold stimulation of the area. The electric responses during the period of stimulation are masked by the stimulus artifacts, quite large under these conditions. The coupling condensers in the amplifier were small, so that the amplifier was blocked only briefly by these large signals. The record is meaningful shortly (about 0.2 sec.) after the end of stimulation. In figure 10 is reproduced a record of a tonic-clonic transcortical response to symmetrical contralateral stimulation taken from the left area 4 arm with the d.c. amplifier. The recording electrodes were chlorided to minimize polarization. The slow components of the electrogram are undistorted, hence the differences between this record and that in figure 9.

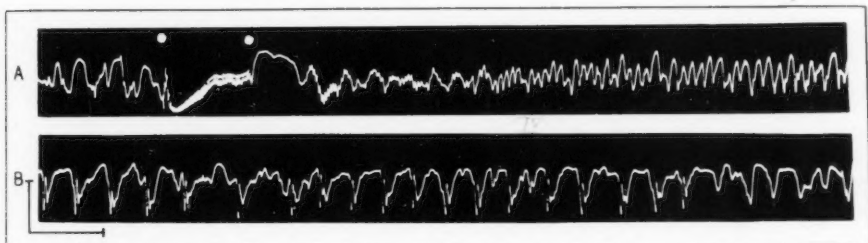


Fig. 10. Transcortical record of a tonic-clonic response of area 4 arm taken with direct-coupled amplification to avoid any distortion of slow waves. The period of stimulation (induction shocks) of the symmetrical contralateral area is shown by the two white dots in A. Interval between A and B: 6 sec. Voltage calibration: 1 mv.

The typical self-sustained cortical response was as follows. Immediately after a brief period of stimulation there was silence in the corticogram. A series of fine, rapid (30 to 18 per sec.) oscillations then developed, increasing progressively in amplitude and often showing "beats" of slow period (see fig. 11). The rapid small oscillations were followed by larger and slower (15 to 6 per sec.) regular waves. An interval of irregular activity of larger amplitude then followed, in which some fast ("spike") and slow components were present. These components then tended to organize as regular rhythmic patterns with a progressively decreasing rate. Each of these patterns consisted of one or more spikes and a slow, large, "round" wave. As the response progressed there was usually an increase of the number of spikes in each burst—from 1 up to 7. The end of the response was sudden and was followed by a uniform electrogram in which the original spontaneous activity was decreased or absent. The spontaneous excursions then reappeared and slowly grew to their resting amplitude.

There was no obvious correlation between the early parts of the cortical elec-

tric responses and the mechanogram of the corresponding muscle. The large initial tonic contraction could begin at the time of relative cortical silence shortly after stimulation. Also the period of muscular quiescence between the tonic and the phasic stages of the mechanical response was not coincident with decrease of cortical activity. This independence of cortical response and motor effects during the early stages of the tonic-clonic sequence is apparent

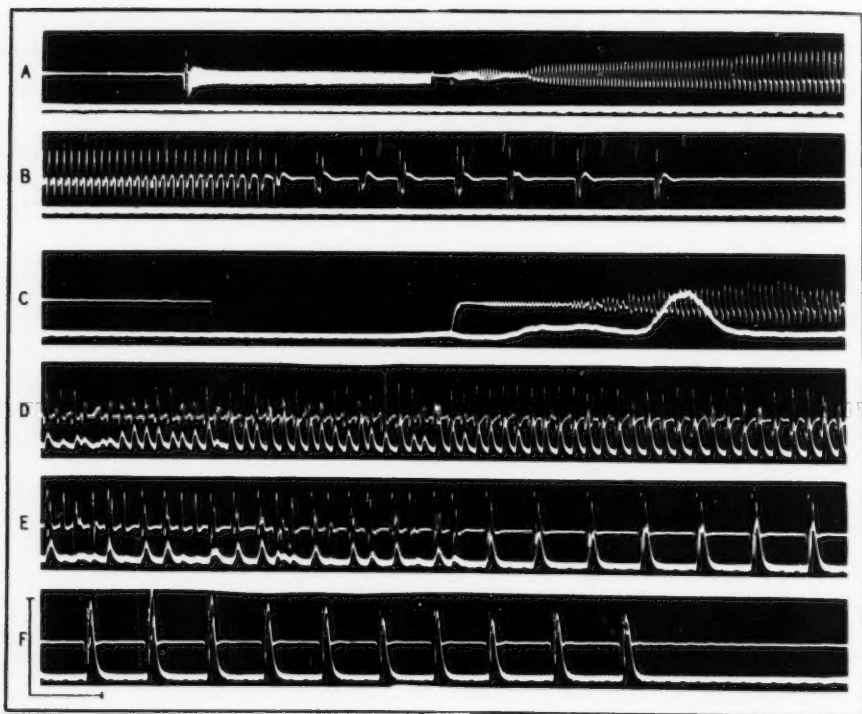


Fig. 11. Tonic-clonic responses from area 4 without and with motor response of the corresponding muscle. The records are as in figure 9. A and B show the cortical response to weak stimulation of the left 4 arm region; the interval between these 2 records is 1 sec. C to F, taken with approximately 2-sec. intervals, show the cortical and muscular responses to simultaneous stimulation of the left 4 arm area, as in A, and of the right 4 arm area. The latter stimuli, when tested alone, were too weak to elicit any self-sustained response at the recording region. Voltage calibration: 2 mv.

in figure 9. It is likely that subcortical centers may add to or subtract from the cortical output and thus determine the activity of the final common path.

During the period of clonic contractions, on the other hand, the rhythmic patterns of the cortical response were correlated in rate and in amplitude with the muscular contractions. When the clonic contractions progressively declined at the end of a response, there was a similar progressive decrease of the

rhythmic cortical phenomena. The more usual gradual increase and sudden abrupt end of the clonic muscular sequence was paralleled by a progressively increasing number of spikes in each of the cortical cycles and by a sudden end of the series.

Although the mechanograms and the corticograms were only correlated during the clonic phase of the responses, for convenience the term "tonic-clonic sequence" will be used also when referring to the cortical self-sustained activity.

b. *Self-sustained tonic-clonic activity in area 4 without movement.* When stimulation of a primary motor point elicits a cortical response this response is attended by appropriate muscular movement. Stimulation of neighboring or of contralateral areas, on the other hand, can produce self-sustained tonic-clonic activity in a given region of area 4 without any contraction of the corresponding muscles (cf. McCulloch, 1937; Moruzzi, 1939). A suitable intermediate intensity, frequency, or duration of the stimuli has to be selected in order to obtain this effect; less stimulation will not cause any indirect cortical activity, although direct effects may be recorded; more stimulation will elicit indirect tonic-clonic movements.

Figure 11 illustrates the phenomenon. The records are as in figure 9 from the left finger flexors and the corresponding primary motor point in the right motor cortex. In A and B stimulation of the left motor arm region with condenser discharges at the rate of 60 per sec. and a selected low voltage elicited cortical activity but no muscular contractions. In C, the same stimuli were applied to the left arm motor region, but in addition the right arm motor area, near the recording electrodes, was simultaneously stimulated with induction shocks of an intensity inadequate to cause any self-sustained activity, cortical or motor, when such shocks were delivered alone. The combination of the stimuli resulted in a corticogram not very different from that in A and B, but it now produced a well-developed muscular accompaniment.

The only differences seen between the cortical responses which evolved without movement and those which were accompanied by mechanical effects were quantitative. There was no specific component of the electrograms absent when there was no movement, and present when movement ensued.

c. *Tonic-clonic activity in other cortical areas than the motor area.* Electrical records, quite similar in their general characteristics to those from area 4, may be obtained by suitable stimulation from other cortical areas than area 4 (cf. Adrian, 1936; Dusser de Barenne and McCulloch, 1938). Tests were made, with positive results, on the following areas: 9, 8, 6, 1, 2, 5, 7, 22, 19, 18 and 17, in monkeys (see figs. 14 to 17). In dogs and cats tonic-clonic effects were readily recorded from any point on the exposed surface of the cerebral hemispheres.

After removal of the neocortex, the hippocampus can be studied both in monkeys and in cats, especially well in the latter where it can be readily isolated from surrounding neocortex. In both species characteristic self-sustained cortical activity was elicited. Figures 12 and 13 illustrate typical responses from the motor area and the hippocampus of a cat. The discharges in the neocortex (fig. 12) were slower than those in the hippocampus (fig. 13). The characteristic

alternation of a sharp quick spike with a slower wave, shown in figure 13, was regularly seen in records from the hippocampus of the cat. These components of the response may be similar to those reported by Renshaw, Forbes and Morison (1940) upon stimulation of nerve fibers from the area entorhinalis, afferent to the hippocampus.

As was the case in area 4 (p. 699), tonic-clonic responses in the other areas mentioned, if sufficiently ample and prolonged, were followed by a complete

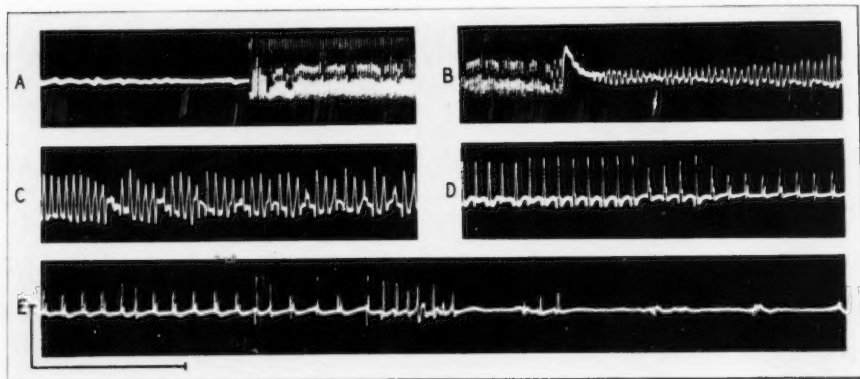


Fig. 12. Tonic-clonic response in the motor area of a cat. Surface electrodes. Approximately 1-sec. intervals separate the successive strips. Voltage calibration: 1 mv.

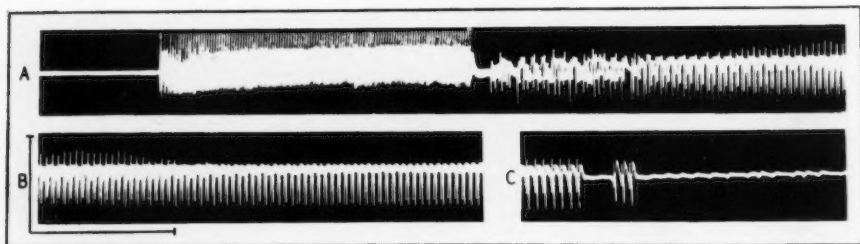


Fig. 13. Tonic-clonic response in the hippocampus of a cat. Surface electrodes. Approximately 3-sec. intervals separate the successive strips. Voltage calibration: 2 mv.

silence in the electrocorticograms. The spontaneous background of activity then slowly built back to its original resting level.

d. *The tonic-clonic activity is a cortical phenomenon.* The cortical responses from the motor areas were practically unchanged when, under artificial respiration, curare was injected in doses sufficient to abolish all neuromuscular transmission. This indicates that afferent impulses from the active muscles are not of importance for the cortical tonic-clonic activity. The inference is further supported by the observations on cortical responses without muscular contractions (fig. 11, A and B).

In several monkeys an occipital pole was isolated from the rest of the cortex and from all subcortical centers by a subpial transection through area 19, externally, and the corresponding regions in the medial and lower aspects of the brain. The completeness of the section was verified at the end of the experiment. Typical tonic-clonic responses were readily elicited in the isolated cortex of area 17.

e. *Spread of cortical activity.* With increasing frequency, intensity and duration of the stimuli applied to a given area the following changes took place. Within that area there were at first only unsustained effects. A greater degree of stimulation led to the appearance of tonic-clonic responses and the amplitude and duration of these responses increased with the stimuli. Initially the tonic-clonic effects were limited to the region stimulated, but with increased stimulation the responses spread to neighboring areas and to contralateral, preferentially symmetrical regions. Figures 14 and 15 illustrate the spread of activity to various cortical areas.

In some animals the spread of activity was mainly ipsilateral, in other animals crossed effects were very readily evoked. The source of this variability was not traced. The general statement can be made, however, that relatively light anesthesia, and, especially, a good condition of the cortex were favorable for the spread of tonic-clonic effects, both ipsi- and contralateral. Widespread bilateral responses were often present shortly after exposure of the cortices, whereas the responses were limited later in the experiment.

As stated before, the crossed responses were in general most prominent in the area symmetrical to the region stimulated. The sensory areas 17, 1 and 2, however, should be mentioned as signal exceptions. Crossed responses from these areas were absent (17) or difficult to obtain (1 and 2).

For the same degree of stimulation more widespread effects resulted from certain areas than from others. It is of course difficult to quantify accurately the degree of spread. Nevertheless, it was obvious that stimulation of areas 6, 4 and 1 resulted in larger diffusion of activity than did stimulation of areas 9, 19 or 17. In addition, it was obvious that the diffusion took place more readily in the backward than in the forward direction.

The spread of tonic-clonic activity was gradual. The ipsilateral areas in the close neighborhood of the stimulated region began their response earlier than did more distant regions. Similarly, in the contralateral hemisphere the effects were more prompt in the area symmetrical to the stimulated point than in other areas. Some of the distant regions frequently did not share in a given response until quite late (up to 3 min.), at a time when the stimulated area was well in the clonic period. In such cases the activity of these distant regions was characterized by a brief initial period of rapid discharges, followed shortly by clonic bursts synchronized with those in the rest of the active cortex. An example of gradual spread of activity is illustrated in figure 16.

In contrast to the marked temporal dispersion which could be seen for the beginning of activity at different areas, the end of a given response was simultaneous for all the active regions in one or in both hemispheres (fig. 16 II).

The two factors emphasized thus far that determine the degree of spread of a response are the characteristics of the stimuli, and the distance of the recorded

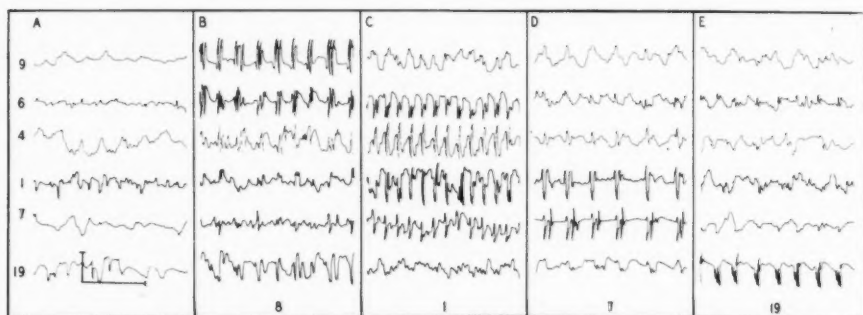


Fig. 14

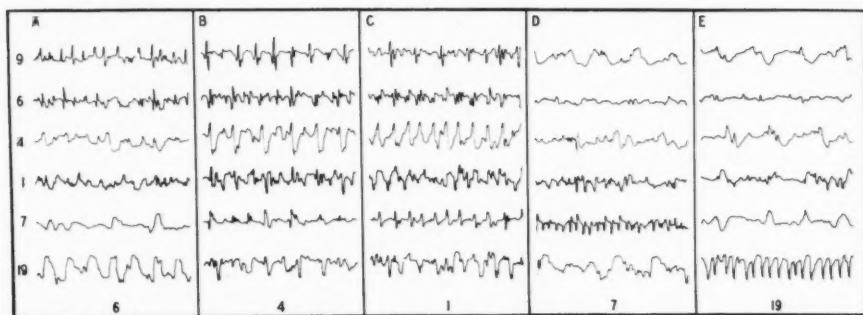


Fig. 15

Figs. 14 and 15. Spread of self-sustained responses to different areas, depending on the region stimulated. Ink tracings of moving-coil galvanometers. The records correspond from above downwards to surface electrodes in the middle region (arm band of Dusser de Barenne and McCulloch, 1938) of the following areas in the right hemisphere: 9, 6, 4, 1, 7, and 19, as indicated in A. The records may be visualized as corresponding to a hemisphere placed with the frontal pole above and the occipital pole below.

Fig. 14A shows the background before stimulation, voltage calibration: 1 mv. The other strips were taken during the clonic period of the responses elicited by stimulation of different areas (see numbers in each of the strips). In figure 14 the stimuli were applied to the right (ipsilateral) hemisphere as follows: B, area 8; C, 1; D, 7; E, 19. In figure 15 the left (contralateral) hemisphere was stimulated as follows: A, area 6; B, 4; C, 1; D, 7; E, 19. It is noticeable that as the stimuli were delivered to progressively more posterior regions of the cortex the peak of activity moved in a parallel fashion.

regions from the stimulated point. The question arises whether preferential connections between different areas may not be significant for the spread. Thus, if a given area A had specific preferential connections with another distant area

B in the same hemisphere, stimulation of A might lead to responses of B, while other regions, closer to A than B, might not share in the response. This question is especially pertinent since Dusser de Barenne and McCulloch (1938) found that local applications of strychnine or electrical stimulation of a given region of the sensory cortex results in a spread of activity to other areas following definite pathways. This preferentially directed spread allows the subdivision of the sensory cortex into transverse bands that include several cortical architectonic

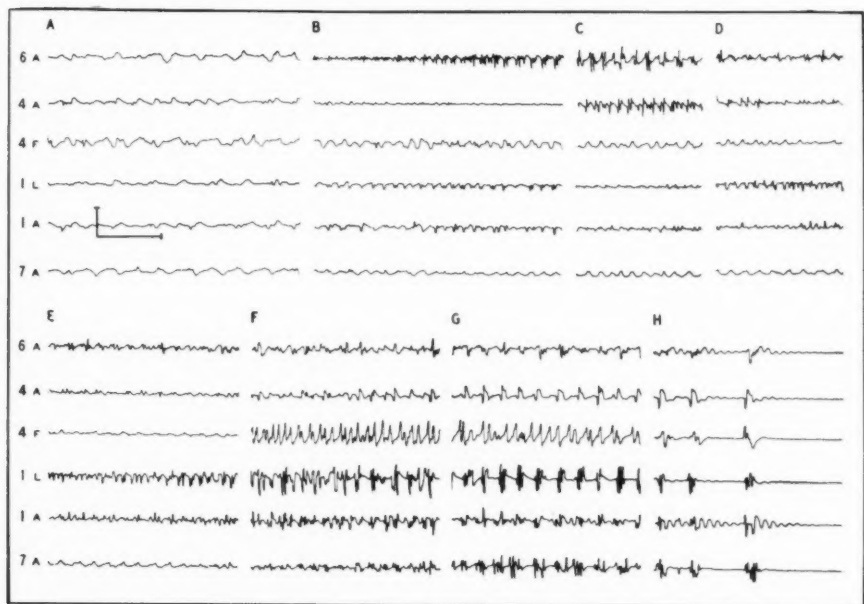


Fig. 16. Gradual spread of a tonic-clonic response. Ink tracings of moving-coil galvanometers. The records correspond from above downwards to surface electrodes on the following areas in the right hemisphere: 6 arm, 4 arm, 4 face, 1 leg, 1 arm, 7 arm, as indicated in A and E.

A shows the background, before stimulation. Voltage calibration: 1 mv. B to H: 3, 8, 12, 20, 30, 40 and 60 sec., respectively, after stimulation of the right area 6 arm for 2 sec.

areas, and that correspond to *leg*, *arm* and *face*, respectively. Thus, application of strychnine to the area 4 arm or electrical stimulation of this area may cause activity of the distant areas 5 arm and 7 arm, in addition to 1 arm and 2 arm, while areas 6 arm, 4 leg and 4 face, although quite close to the stimulated 4 arm, may show no activity over their normal background. Similarly, a preferential facilitating action on the chewing area of the rabbit was found by Moruzzi (1939) upon stimulation of the acoustic area; stimulation of other equidistant points had no such facilitating effect.

The following experiments were planned to test the influence of preferential

connections as opposed to mere distance between recording and stimulated areas. Recording electrodes were placed at different *leg*, *arm* or *face* levels in different areas. For instance, the 6 pairs of electrodes could be on 6 *arm*, 4 *leg*, 4 *arm*, 4 *face*, 1 *arm* and 7 *arm*. Stimulation was then applied to the *leg*, *arm* or *face* subdivisions of the areas under consideration and the degree of spread was examined. Although occasionally a preferential spread along a transverse axis (*leg*, *arm* or *face*) was encountered, in general the most important factor which determined the spread in one hemisphere was proximity to the stimulated region. Thus, in figure 17B stimulation of 7 *leg* resulted in marked clonus of 7 *arm* but not of 1 *leg*; in C, stimulation of 7 *face* caused maximal effects in 7 *arm*, and affected equally 1 *arm* and 1 *face*; in D, the effects of stimulation of 1 *leg* are prominent both in that area and in 1 *arm*; in E, the spread of activity from 1 *arm* is mainly to 1 *leg* and 1 *face*, rather than to other arm regions; finally, in F, stimulation of 1 *face* caused responses of that area and also of the arm regions, the more marked the shorter the distance to the stimulated area.

The importance of the distance factor within a given area was tested as follows. The arm motor area (4 *arm*) on one side was mapped with threshold stimuli. Three parallel pairs of recording electrodes were placed within that area. Stimuli were then applied above (4 *leg*) or below (4 *face*) the recording region. By selection of the stimuli it was possible to show that the tonic-clonic activity of the area was maximal near the stimulated region. A similar procedure yielded similar results in area 4 *face*. Figure 18 illustrates these observations.

The discrepancy between the results illustrated in figures 17 and 18 and those reported by Dusser de Barenne and McCulloch (1938) was interpreted as due to differences in the experimental conditions. In order to clarify these differences we had the advantage of Dr. W. S. McCulloch's collaboration for three days in our Laboratory. The conclusion was reached that the significant difference of procedure lay in the anesthetic. Whereas Dusser de Barenne and McCulloch used dial, the present experiments were carried out under chloralose anesthesia. Two monkeys were studied during McCulloch's stay here, one under chloralose, the other under dial, and the observations confirmed that conclusion.

f. *Synchronism. • Pace-makers.* The self-sustained response of a given cortical area was well synchronized for different regions within it, both during the tonic and the clonic periods. The features in records from several pairs of electrodes applied to the area were quite simultaneous.

When several areas in one or both hemispheres shared in a response, asynchronism was the rule for the early part of the tonic activity. Quite different rates of discharge could then be seen at the different regions. Synchronization of activity usually took place, however, later in the tonic period and invariably during the clonic discharges. Figures 14 to 16 show this synchronization in quite distant areas of the same or both hemispheres. Careful measurement of the time for the onset of the corresponding discharges indicates that such discharges occur within no more than 50 msec. at all the areas involved.

This remarkable correlation in time of the responses at distant areas suggested that some region, possibly the stimulated region, was setting the pace of the gen-

eralized clonic activity. That region would then be the pace-maker for the response. The following observations demonstrate that this suggestion is wrong. In some experiments the stimulated area was rapidly excised as soon as generalized clonus had appeared. This excision did not modify significantly either the rate or the amplitude and duration of the response in the remaining active cortex (see Bubnoff and Heidenhain, 1881; François-Franck and Pitres, 1883).

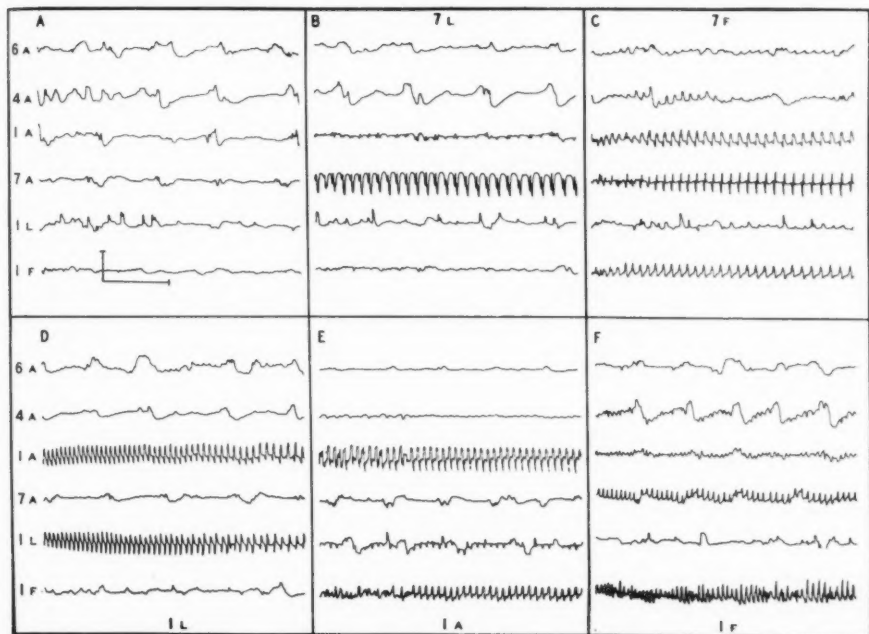


Fig. 17. Spread of tonic-clonic responses as a function of distance from the area stimulated. Ink tracings of moving coil galvanometers. The records correspond from above downwards to surface electrodes on the following areas in the left hemisphere: 6 arm, 4 arm, 1 arm, 7 arm, 1 leg, 1 face, as indicated in A and D. Voltage calibration: 1 mv.

A shows the background, before stimulation. B to F were taken during the clonic period of the responses corresponding to stimulation of the following areas of the same hemisphere: B, 7 leg; C, 7 face; D, 1 leg; E, 1 arm; F, 1 face (see numbers in each of the strips).

The measurements of the time at which the clonic bursts started in different areas showed a variability in the order of their appearance. A given area could come in shortly before or shortly after another area; and the stimulated region did not always lead (cf. Adrian, 1936).

In some experiments 4 electrodes were placed in a square having sides of about 1 cm. on the surface of a cortical area. Records were taken from the 6 combinations by pairs which the electrodes provided (the 4 sides and the 2 diagonals of the square). This method of recording allowed investigation of the direction

from which conducted waves from another region would reach the area. A changing polarity in the diphasic responses consistent with the direction of the moving wave would determine this direction. The areas neighboring each of the sides of the square were then stimulated successively. No consistent polarity of the responses was seen when these responses were fairly generalized.

It may be inferred, therefore, that there is no systematic pacemaker in generalized cortical responses. The active areas become coupled so that a discharge in one is followed instantly by discharges in the others. But any of the regions

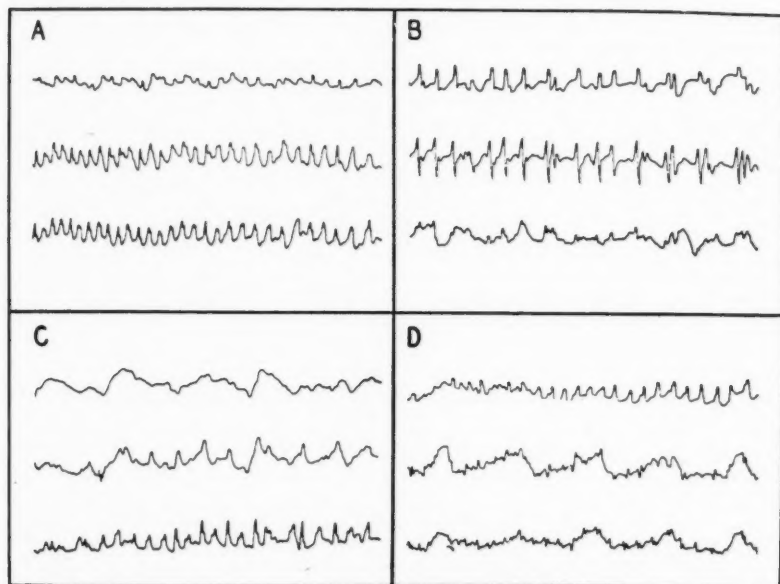


Fig. 18. Spread of tonic-clonic responses as a function of distance from the stimulated point. Moving coil galvanometer tracings. The records for A and B are from 3 pairs of electrodes placed parallel on the area 4 arm. The uppermost pair was close to the area 4 leg and the lowermost close to 4 face. A was taken during the clonic stage of a response to weak stimulation of 4 face; B, during the response to stimulation of 4 leg; C and D illustrate similar responses to stimulation first "below" and then "above" with the recording electrodes placed on area 4 face.

involved may trip the coupled system. The situation is analogous to that which would take place in a heart in which all regions would have similar time constants. The impulse would start in different places at random and there would not be any specific pace-maker.

g. Rates. The previous analysis of the pace-makers of cortical activity leads to the inference that the intrinsic rate for self-sustained responses is approximately the same for different areas throughout the cortex. This was found the case when discrete—i.e., not spreading—responses were elicited by moderate stimulation of different regions.

Several frequency-time curves were plotted for the responses of various areas. Characteristically they all showed two breaks. The rate first declined slowly, then dropped suddenly to another lower level. A second slow decline was again followed by a sudden drop to a still lower level. This rate then dropped only slightly till the end of the response. As will be shown later the responses consist of a sequence of different patterns or components, which probably correspond to discharges in different cortical elements (see section *i*). According to this interpretation the frequency-time curves may not be homogeneous throughout a response—i.e., they may not describe the changes of frequency in the same elements. Indeed, it is interesting that the two breaks in the curves corresponded to the transition first from component I (section *i*) to component II, and later from component II to III and IV (clonus).

If the frequencies corresponding to each of these components are considered separately, then component I has a range of from 30 to about 18 per sec., component II from 14 to about 7 per sec., and finally, the clonic bursts from 3 to 1 per sec. These rates were found in all the areas of the monkey studied, and also in all the responses measured, whether brief or long, localized or widespread. It may be inferred, therefore, that, unlike the degree of spread and the amplitude and duration of the responses, all of which increase with the degree of stimulation, the rate of the responses is independent of the intensity, frequency or duration of the stimulus applied.

The cortical clonus, like the motor clonus (fig. 1B), could show sudden changes in rate. Occasionally one area apparently discharged at exactly twice the rate of another area (fig. 19), but such records may be interpreted as revealing the presence of an additional component in the faster area, absent in the slower regions.

The end of the responses corresponded as a rule to a slight slowing of the clonus. Thus, the rate of the clonic bursts, quite regular for several seconds at about 2 per sec., could decrease to 1.5 or 1 per sec., whereupon the response abruptly ended. Occasionally a few isolated irregular clonic bursts followed a brief (about 2 sec.) silent period.

h. Facilitation and inhibition. A summation of the effects of stimulation of two areas was readily demonstrated, as follows. Frequencies, intensities and durations of stimulation of one of several areas (e.g., *G*, *4 leg* or *1 arm*, in either hemisphere) were determined which did not cause a tonic-clonic response in a test area (e.g., *4 arm*). Simultaneous stimulation of two of those areas could then elicit typical responses in the test area. The records in figure 11 show the facilitating influence of subliminal stimulation of a motor area on the motor responses to stimulation of the opposite symmetrical area.

A summation was also seen of the effects of repeated stimulation of a given area. Thus, if brief repetitive trains, inadequate singly to elicit self-sustained activity, were repeated at intervals of 1 to 5 sec., a tonic-clonic sequence could result after application of a few trains. Continuation of the stimuli during the tonic-clonic response then resulted in brief periods of abolition of the clonic activity, following the direct effects of the stimuli. During this period of de-

pression the clonic bursts were substituted by rapid small waves, similar to those which occur at the beginning of a response (component I). The results described here resemble those illustrated in figures 6 and 7 for motor responses.

As already stated (p. 702), a tonic-clonic response was followed by inhibition of the spontaneous activity of the corresponding area. This inhibition was maximal at the stimulated point. It was more marked after prolonged than after brief responses.

i. *Different components in the electric responses.* In such complex records as those illustrated in figures 9 and 10 it is desirable to systematize some of the

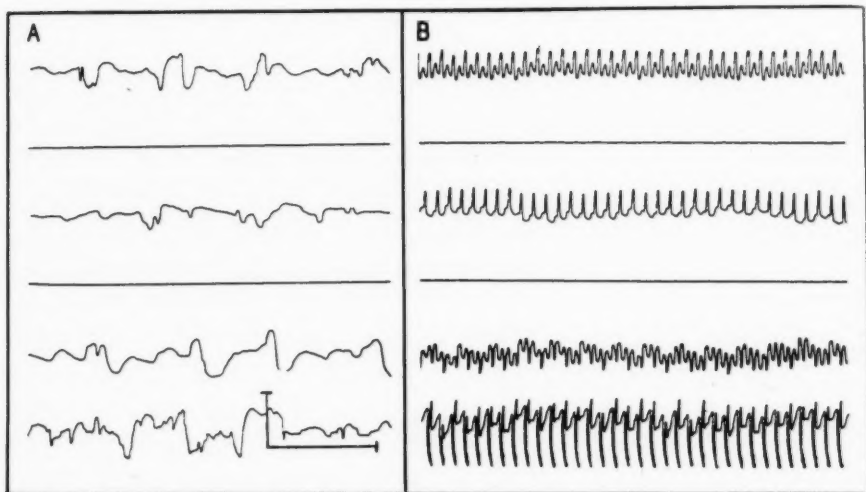


Fig. 19. Synchronism of responses in various areas. Moving-coil galvanometer tracings. Records from above downwards; surface electrodes on the left area 4 leg; needle electrodes in a right leg muscle; surface electrodes on the left area 4 arm; needle electrodes in a right arm muscle; surface electrodes on right area 4 leg; surface electrodes on right area 1 arm.

A, background before stimulation. Voltage calibration: 1 mv. B, during the early clonic stage of a response to stimulation of the right area 4 arm. Disregarding alternation, the rates of the waves in areas left 4 leg and right 4 leg are twice those in areas left 4 arm and right 1 arm.

waves encountered for purposes of identification and description. On the basis of amplitude, frequency and phase, the following components may be recognized.

Type I (fig. 20A) consists of rapid (30 to 18 per sec.) small sinusoidal waves. Their amplitude, whether recorded by surface electrodes 3 to 10 mm. apart, or by an inserted and a surface electrode about 3 mm. apart, was from 0.1 to 0.5 mv. They usually were prominent at the beginning of the responses (figs. 10 and 11). They grew slightly and slowed as the response progressed.

Type II (fig. 20B) is a simple, regular, elongated excursion. It was frequently diphasic when recorded with electrodes on the surface, and monophasic when

recorded transcortically, from the surface to an inserted electrode. Its frequency was from 15 to 6 per sec.; its amplitude from 0.4 to 1.0 mv.; its duration (per wave) from 60 to 150 msec. Although it might be interpreted as a larger and slower aspect of the component I described before, it has been distinguished as a separate component because the transition between the two is usually fairly sudden and because II shows only rarely the slow rhythmic changes of amplitude (beats) which are common in I.

Type III is a sharp, brief (10 to 80 msec.) spike-shaped excursion (fig. 20C, D and F). These, like II, could be diphasic (surface electrodes) or quite purely monophasic with the surface positive (transcortical records). Their amplitude

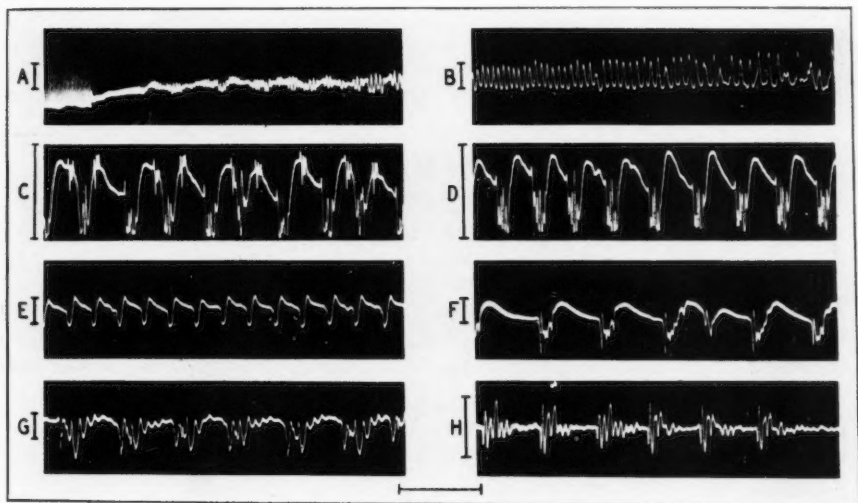


Fig. 20. Different components of the cortical self-sustained responses. A and B are from surface electrodes in area 19; C and D are transcortical records from the same area; E to H are from area 4 arm; E and G are surface; F and H, transcortical records. Voltage calibrations: 1 mv.

was from 0.5 to 3 mv.; they were the largest excursions recorded. Their frequency in an individual clonic burst in the monkey's neocortex could reach 20 per sec. Even higher frequencies (up to 45 per sec.) were recorded from the cat's hippocampus.

Type IV is a large (0.5 to 2 mv.) rounded wave (fig. 20C, D, E and F). It could appear diphasic or monophasic in the records. It was usually associated with the appearance of spike (type III) components which obscured its characteristics. Its frequency varied from 3 to 1 per sec.

Type V resembles a train of damped oscillations (fig. 20G and H), of rapid frequency (10 to 20 per sec.) and moderate amplitude (from 0.1 to 0.5 mv.).

The clonic bursts consisted typically in all regions of the cortex of one or more

spikes (component III), followed by or superimposed on a large rounded wave (component IV). The records frequently had the "spike-dome" appearance which Gibbs, Davis and Lennox (1935) have described as characteristic of petit-mal epilepsy in human subjects. Only exceptionally were any spikes apparent late in the rounded course of component IV. Figure 20 C illustrates one of these exceptional instances; as shown in D the clonic bursts later in the same response reverted to the usual type. In area 4 the clonic bursts consisted of the typical III-IV combination, but in addition they were often followed by component V.

The relative independence of the several components described was indicated by the possibility of obtaining responses exhibiting exclusively one of them. Thus, although the usual sequence in the tonic-clonic activity was first I, then II and then III, IV and occasionally V during the clonic bursts, responses consisting only of I, or of I followed by II, were not uncommon. In areas distant from the stimulated region the response could consist mainly of clonic bursts involving III and IV, with only a brief initial component I. This independence

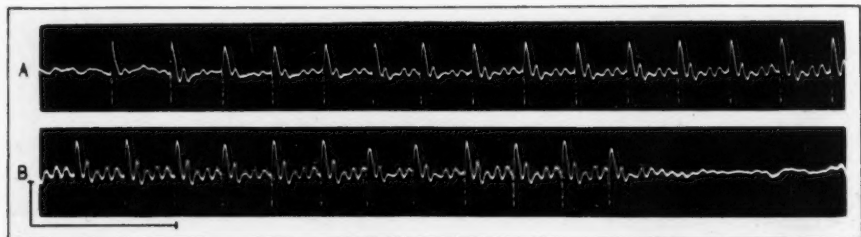


Fig. 21. Progressive build-up of self-sustained component V upon repetitive stimulation at a frequency of 3 per sec. Transcortical record from the left area 4 arm. Stimulation of the left area 4 leg. Voltage calibration: 2 mv. B is the continuation of A.

was further emphasized by the effects of single shocks or of brief repetitive trains of stimuli delivered during a response. These shocks increased or favored the appearance of I and V (fig. 21), while they inhibited II (fig. 33).

The first four components were found in all the neocortical areas tested. Component V was found mainly in area 4, and less frequently in the adjacent areas 1 and 6. In area 4 this component could be readily built up without any of the others by repeated application of single shocks at slow frequencies (fig. 21).

C. *Unsustained Cortical Responses.* a. *Responses to single shocks.* Stimulation of any region of the cortex with single shocks led to the appearance of electric responses in both the ipsilateral (see Adrian, 1936) and contralateral (see Curtis, 1940a) hemispheres. These responses varied in latency, amplitude and phase with the mode of recording adopted—i.e., they were different when two surface electrodes were used and when they were led off transcortically, from one electrode on the surface to another inserted beneath the gray matter. With similar arrangement of both the stimulating and recording electrodes the responses differed at various cortical areas. Certain general characteristics, however, may be described as follows.

The responses of the ipsilateral hemisphere were maximal in the close vicinity of the region stimulated. Increasing the distance between the stimulating and the recording electrodes produced the following changes. The responses declined in amplitude. The latency and the time to peak increased. The records were prolonged. Figure 22A illustrates these changes.

The prolongation of the responses with increasing distance may be interpreted as denoting temporal dispersion. In agreement with this interpretation are the results of stimulating the contralateral hemisphere. The responses were

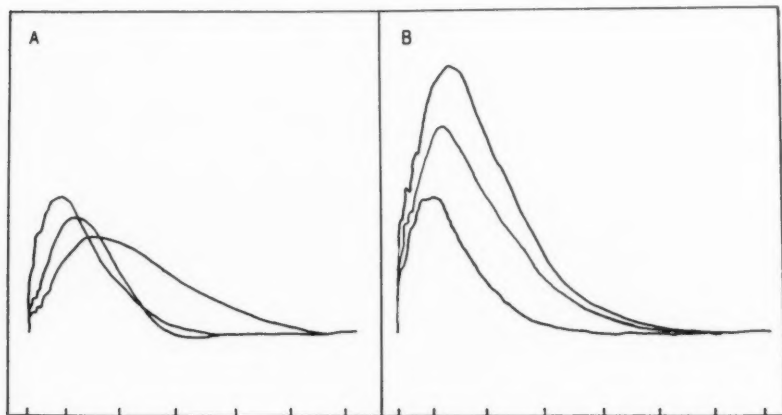


Fig. 22. Influence of the distance between the stimulating and the recording electrodes (A), and of the distance separating two surface recording electrodes (B), on the responses to single shock stimulation. The stimulating electrodes, 3 mm. apart, were placed transversally on area 1 *leg*. Four recording electrodes (1 to 4) were placed longitudinally in line below the stimulating pair, at distances of approximately 3 mm. The stimuli were constant for all the records. The figure was made by projecting the original pictures through a photographic enlarger and then tracing them after superposition. Time scale: 2 msec. The waves indicate positivity of the lead proximal to the stimulated point.

A. Responses recorded from electrodes 1 and 2 (upper tracing), 2 and 3 (middle tracing), and 3 and 4 (lower tracing).

B. Responses recorded from electrodes 1 and 4 (upper tracing), 1 and 3 (middle tracing), and 1 and 2 (lower tracing).

then more prolonged than those obtained from the ipsilateral side and this longer duration became more prominent when first the area symmetrical to the record was stimulated, and then other areas.

The response to weak (just threshold) stimuli was usually a relatively simple monophasic wave. As the stimuli were intensified this wave increased in amplitude and, in addition, new waves appeared, superimposed upon, earlier or later than the original deflection (figs. 23 and 24A). That these several components were due to different sets of responding cortical elements was shown by the analysis of the polarity of the components (to be described below), by the independence of the changes in these components upon repetitive stimulation (fig. 29)

and by the observation that the degree of spread over the cortex varied for the different waves. As a rule the low threshold components spread further than the higher threshold responses (fig. 25).

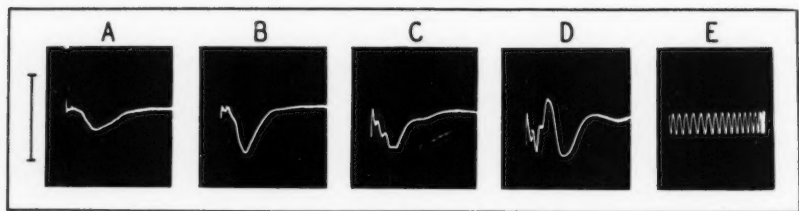


Fig. 23. Progressive appearance of additional components in the responses to single stimuli when the shocks are gradually intensified. Stimulating electrodes on the upper part of the left area 17. Surface recording electrodes 3 and 7 mm., respectively, from the stimulating cathode. The shocks were discharges of a $0.04 \mu\text{F}$ condenser. The discharge circuit included resistances of $5,000 \omega$ in series and $5,000 \omega$ in parallel with the cortex. Upward excursions in the records denote positivity of the lead proximal to the stimulated region with respect to the distal lead. Voltage calibration: 1 mv.

The voltages of the shocks were: A, 5; B, 8; C, 12; and D, 20 v. E: 500 cycles.

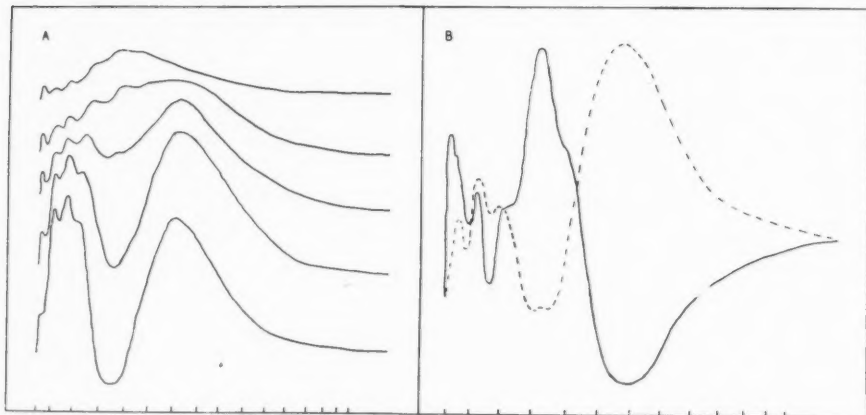


Fig. 24. A. Transcortical responses to single shocks of progressively increasing intensity. The records were taken as in figure 23, but the recording electrodes were one on the surface, the other inserted 2.5 mm. deep. Upward excursions denote negativity of the cortical surface with respect to the deep layers. The successive tracings from above downwards correspond to shocks with the following intensities: 10, 15, 20, 30 and 40 v. Time scale: 2-msec. intervals.

B. Responses of area 17. The records were taken from fixed surface electrodes. The stimuli were delivered from above (medially, solid line) or from below (laterally, broken line) the recording leads. For explanation see text. Time scale: 2-msec. intervals.

The distance between the two recording electrodes markedly influenced the amplitude of the responses. The observations were made with the stimulating electrodes and the nearer recording electrode in fixed positions. As the farther

lead was shifted away from the nearer one the responses increased in amplitude. Figure 22B illustrates a typical observation. It may be inferred that the cortical potentials sum in series, a condition similar to that found in some smooth muscle systems (Rosenbluth, Davis and Rempel, 1936).

b. *Responses of areas 4 and 17.* Characteristically the response from area 4 to shocks of moderate intensity, recorded by means of surface leads, was a monophasic round wave showing a series of spikes in the initial part of the wave (fig. 25B). Strengthening the shocks resulted in an increase of the amplitude of this

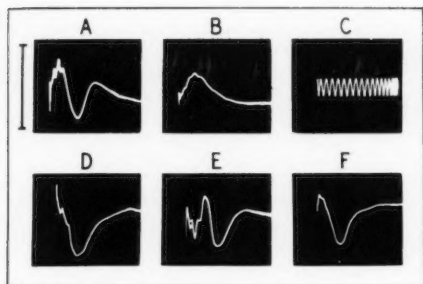


Fig. 25

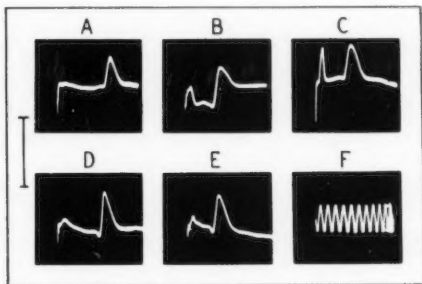


Fig. 26

Fig. 25. Differential spread of the several components of responses to single stimuli. The shocks were applied to area 4 in A and B and to area 17 in D, E and F. The lead-off electrodes were three surface needles (1 to 3) in both areas, placed in line with the stimulating cathode at distances of about 4 mm. A shows the response to a given shock recorded from electrodes 1 and 2 in area 4. B is the response to the same stimulus, recorded from electrodes 2 and 3. D is the response at 1 and 2 in area 17 with a weak stimulus. E shows additional components with the same leads when the shock was strengthened. F was the response at 2 and 3 to the same stimulus as in E. C, 500 cycles. Voltage calibration: 1 mv.; this calibration applies to all the records except to E, which was taken with about one-half the amplification used for the other records. Upward excursions denote positivity of the lead proximal to the stimulating cathode.

Fig. 26. Comparison of surface with transcortical records. Stimuli applied to area 17. A, B and C were recorded with surface electrodes, D and E from the surface electrode proximal to the stimulus to a needle inserted 3 mm. deep. Upward excursions denote positivity of the proximal surface electrode in A, B and C, and negativity of the surface in D and E. F, 100 cycles. Voltage calibration: 1 mv. The intensity of the stimuli was: A, 15; B, 30; C, 40; D, 15; and E, 30 v.

response and in a decrease of its latency. Occasionally strong shocks elicited a second component which appeared as a large wave starting toward the peak of the first component but having an opposite polarity (fig. 25A).

In figure 23 are illustrated typical responses of area 17 to shocks of increasing intensity. Here again the lowest threshold response was a monophasic wave. Stronger shocks decreased the latency of this wave and in addition caused the appearance of earlier, faster components. With even stronger stimuli two late components of opposite polarity appeared in succession, the first opposite in polarity to the original low threshold wave. These two late components, when

both were present, recorded as a large diphasic wave which masked the low threshold wave. With the available data it is not possible to decide whether or not any of these components are equivalent to the 4 studied by Bishop and O'Leary (1936) in responses to stimulation of the optic nerve.

c. Polarity and orientation. The polarity of some of the components mentioned was as follows. Recording from area 4 with 2 surface electrodes parallel to the central fissure, e.g., at the arm area, and stimulating from a more medial region, e.g., from the leg area, the electrode proximal to the stimulated region went positive with respect to the distal electrode during the development of the first low threshold response (fig. 25B). With a similar arrangement of electrodes on area 17 the polarity of this component was reversed—i.e., when the stimuli were weak the proximal electrode was negative with respect to the distal (fig. 25D).

When the record was transcortical, from surface to depth, the surface was positive during the development of the first component in area 4, and it was negative in area 17. It might be concluded that in a given response the surface

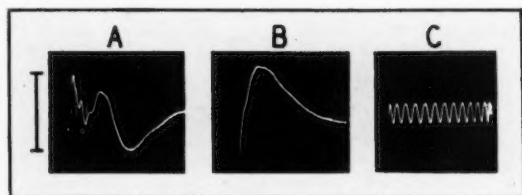


Fig. 27. As in figure 26, but in another animal. A, surface record. B, transcortical record. C, 500 cycles. Voltage calibration: 1 mv.

is positive or negative with respect both to distant points on the surface and to the deep cortical layers. This conclusion, however, is only valid for the conditions described; discrepancies arise when the tests are further elaborated. Thus, some of the components may have different polarities than those mentioned when the surface records are compared with the transcortical responses. In figure 26 are shown early and late responses in area 17 in which the surface lead near the stimulated point was positive with regard to a distant point, while it was negative with regard to an electrode on the underlying white matter.

Further discrepancies appeared in other instances with respect to the several components of a given response. Thus, in figure 27, while the surface record shows the presence of several components of different polarity, the transcortical record appears as a relatively simple monophasic wave.

In some observations the stimulating electrodes were in a fixed position. The records were taken first from an electrode on the surface, then from an electrode inserted immediately beneath the first one, each lead referred to a more distant surface electrode. Such records allowed a comparison of the surface with the deep effects at a given point in the cortex. The results were irregular. While some of the components could in some animals have the same polarity whether

recorded from the surface or recorded from the deeper cortical layers, in other instances the polarity was reversed.

Both in the observations just reported and in those in which transcortical recording was used, the mode of recording could modify differentially not only the polarity, but also the amplitude of some of the components of a response. Such differential changes of amplitude probably indicate that the orientation of the elements involved in the appearance of a given component may be parallel or perpendicular to the surface of the brain.

In other experiments the recording silver needle electrodes were first on the surface. Both electrodes were then inserted into the cortex to depths of from 1 to 4 mm. Typical results are illustrated in figure 28. There was usually a decrease of the amplitude of the recorded responses, but no change in polarity for any of the components.

In a series of observations the recording electrodes were in a fixed position, while the stimuli were delivered first beyond one, then beyond the other of the

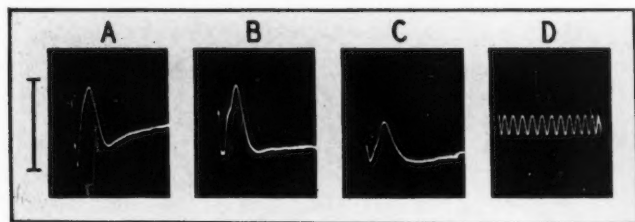


Fig. 28. Responses recorded from area 17 first with two surface electrodes (A), then with the electrodes inserted 1 mm. below the surface (B), and finally with the electrodes inserted 3 mm. (C). D, 500 cycles. Voltage calibration: 1 mv.

recording contacts. For instance, the recording leads were applied to the arm region in area 4 and the stimuli were delivered to the leg or the face regions. The purpose of these observations was to see whether the polarity of the responses would be modified by the position of the stimulated region relative to the recording electrodes. Thus, if a cortical response had a consistent polarity with respect to the stimulated point, e.g., if in the response the proximal electrode were always positive with regard to the distal, then the records from stimulation first above and then below would be mirror images. If, on the other hand, the important factor in determining polarity were the orientation of the elements between the leads rather than the region stimulated, then the records would not reverse when the stimuli were moved.

In general (fig. 24B) the slow components of the responses were reversed in the records when the stimuli were changed—i.e., the polarity of these responses with respect to the stimulated region was constant. Occasionally, however, this polarity was reversed. The early fast components of the responses in area 17 did not reverse in the records—their polarity was independent of the region stimulated.

When the recording electrodes were placed transcortically, instead of on the surface, the polarity of the responses was uninfluenced by changing the region stimulated.

d. *Propagation velocity.* The cortical responses to single shocks spread around the stimulated point. In cats, Adrian (1936) found that the rates of

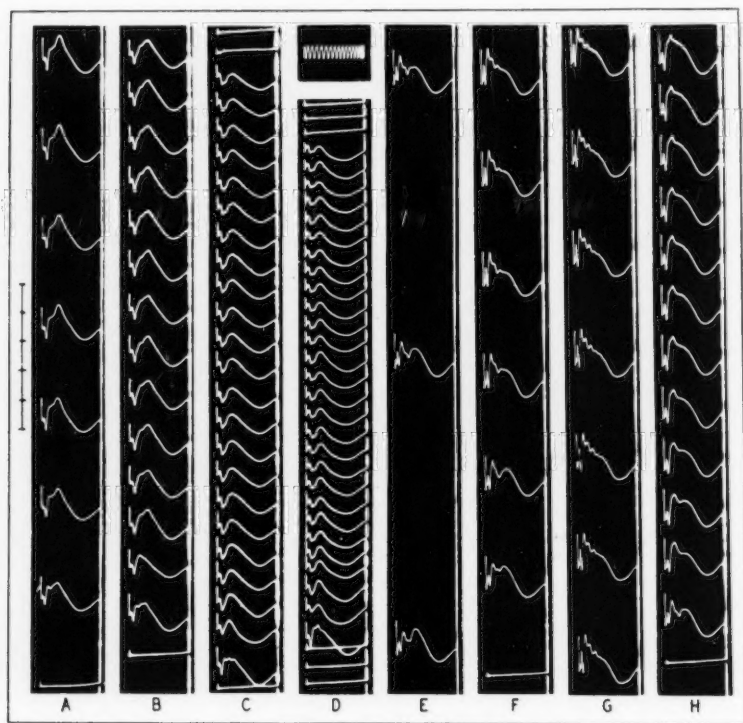


Fig. 29. Influence of frequency of stimulation on the responses of area 17. The records are from surface electrodes. Voltage calibration: millivolts. The time calibration is at the top of strip D, and shows 500 cycles. Each strip shows from below upwards the responses to successive shocks. A to D, stimulation with shocks of moderate intensity with the following frequencies: A, 1.3; B, 3.0; C, 5.0; and D, 7.5 per sec. E to H, stimulation with stronger shocks with the following frequencies: E, 0.45; F and G, 1.3 (G is the continuation of F); and H, 2.7 per sec.

conduction of the "deep" (see discussion) response usually ranged between 25 and 35 cm. per sec. The conduction velocity slowed upon repetitive stimulation. The fastest rate observed was 60 cm., the slowest 5 cm. per sec.

In the present study the speed of propagation of some of the waves was determined by measuring their latency when recorded at various distances from a point stimulated at relatively long intervals and with constant shocks.

The rates of propagation varied for the different components. Thus, the low threshold, relatively smooth, prolonged wave which appears in areas 4 and 17 (fig. 25B and D) spreads with a rate of about 3 m. per sec. (2.3 to 3.7 in different observations). The rate was the same in areas 4 and 17, although the polarity of this wave is opposite in these two areas (fig. 25). The late component occasionally encountered in area 17 (fig. 26) propagated with a rate of only 0.2 m. per sec.

Although the amplitude of the responses decreases with the distance from the stimulated point (fig. 22A), there was no evidence of a corresponding decrease in the rate of propagation—i.e., this rate was constant at the several distances tested (3 to 12 mm. from the stimulated point). Adrian's (1936) observation, that the rate of spread slows with repetitive stimulation, was confirmed.

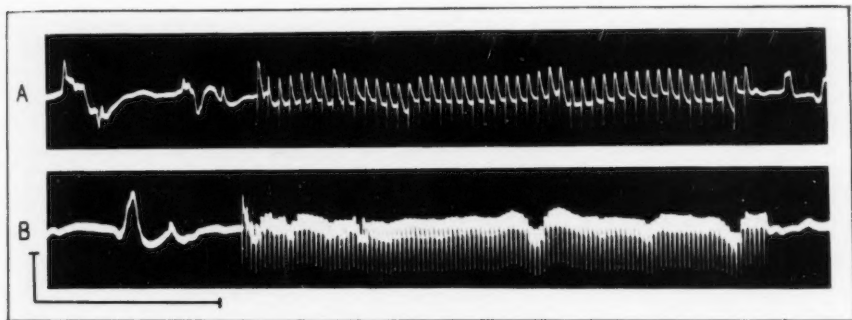


Fig. 30. Responses of area 4 to repetitive stimulation. The stimuli were delivered to the right area 4 arm and the record (surface) was taken from the corresponding area on the left side. Frequency of stimulation: A, 18, and B, 40 per sec. Voltage calibration: 1 mv.

e. *Repetitive stimulation.* The effects of repetitive stimulation at various frequencies were qualitatively similar in areas 4 and 17. Figure 29 illustrates typical results in area 17. The following changes took place. When stimulation was repeated the latency of the several components in the records increased. This increase is interpreted as due at least in part to slowing of propagation (Adrian, 1936). The amplitude of the several components either increased or decreased, depending upon the stimulation rate. The changes in amplitude were independent for the different components—i.e., at a given frequency some could increase while others decreased. Thus, in figure 29E and F the early part of the electrograms increases in the successive responses, while a late component decreases. Quite commonly alternation of the amplitude of successive responses was seen (fig. 29D, responses to the 3rd to 8th shocks; the alternation, striking in the original record, is minimized in the reduced reproduction). Quite commonly also the responses first decreased in amplitude and later increased, although not up to the initial level (fig. 29B). With relatively high frequencies of stimulation (26 to 60 per sec.) the amplitude of the responses promptly (even

at the second shock) fell to a low level and remained small throughout the period of stimulation. Some of the components could, however, be readily identified at frequencies of 40 per sec. (fig. 30).

f. *Facilitation and inhibition.* The increments of amplitude of some of the

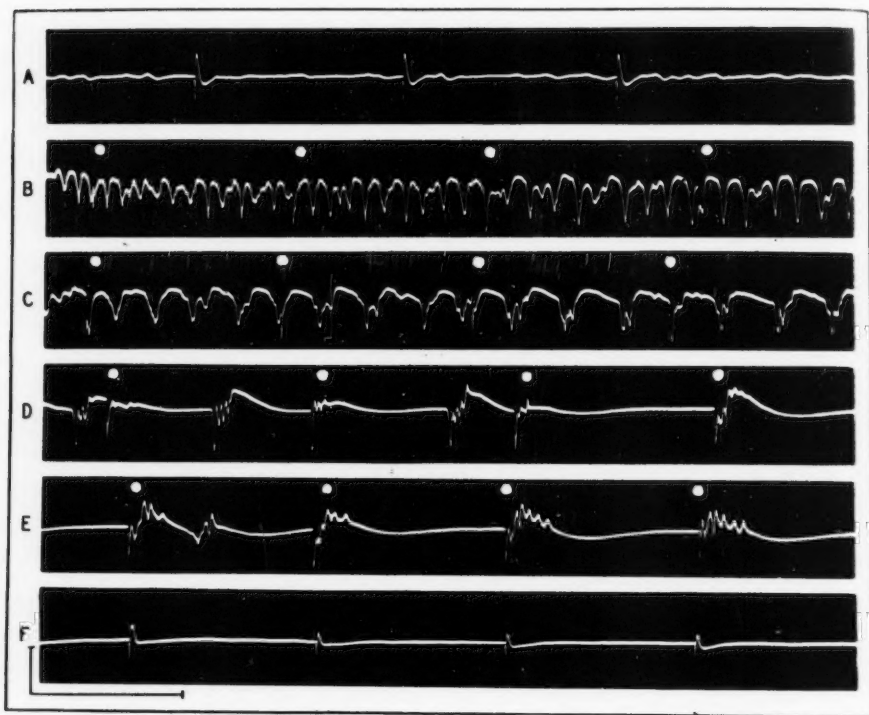


Fig. 31. Influence of a tonic-clonic response on the responses to single shock stimulation. Transcortical record from the left area 4 arm. Voltage calibration: 2 mv.

A shows the responses to single shock stimulation of the left area 4 leg before the tonic-clonic response. The single stimuli were applied throughout the observation at the rate of 0.8 per sec. B, C and D, were taken approximately 5, 10 and 15 sec., respectively, after repetitive stimulation of the left area 4 face had elicited a widespread tonic-clonic response. The dots indicate the delivery of the single stimuli during the period of self-sustained activity. E was taken immediately at the end of the tonic-clonic response, and F, 5 sec. later.

components of a response upon repetitive stimulation at adequate frequencies (fig. 29F) may be interpreted as examples of temporal facilitation. Whether or not the decrement seen at other frequencies was due to inhibition is open to question. Under the terms "extinction" and "suppression" Dusser de Barenne and McCulloch (1939, 1941) have described two special types of cortical inhibition. Extinction is the cancellation of motor response from a given cortical element

due to previous activity of that same element. Suppression is a decrease of responsivity of a certain area (e.g., 4) caused by previous stimulation of specific cortical regions (e.g., 4 *strip*). The depressions of response in figures 29 and 30 are not due to extinction, because extinction follows a period of facilitation, i.e., it is more prominent when long, rather than short intervals separate the stimuli, whereas the decrease of response in these observations was more prominent the faster the frequency of stimulation—the briefer the intervals separating the shocks. Nor are these depressions due to suppression, because they appeared upon stimulation of any cortical region, not of specific inhibitory areas. The decline of response at high stimulation frequencies may be due to an inhibitory action of a type different from extinction or suppression; or it may be due to fatigue of some elements; or, finally, it may depend on refractoriness.

In a series of observations the responses to single shock stimulation of a cortical area with a slow frequency (0.5 to 2 per sec.) were recorded before, during and after a tonic-clonic sequence induced by adequate repetitive stimulation of the same or of another area. During the period of repetitive stimulation and at the beginning of the tonic stage, the responses to the single shocks could be either increased or decreased, depending upon the intensity, weak or strong, respectively, of the repetitive stimulus. They were augmented during the clonic period and for some time after the end of the clonic response. Indeed, at that time the single shocks could elicit complex responses similar to the clonic bursts (fig. 31). This period of augmentation was followed by a prolonged depression in which the responses to single shocks could be almost entirely abolished. A slow recovery to the original level ended the changes brought about by the repetitive stimulus. Figure 31 illustrates a typical instance.

In some observations the cortical record was taken from the primary motor point of a recording muscle. The periods of augmented cortical response to single shocks could then be correlated with the appearance or the increase of muscular responses. Motor responses of the right arm muscles could be readily obtained in these conditions by single shock stimulation not only of the left motor cortex but also of the right area 4 and of the left or right areas 6, 1, and even 7. Figure 32 illustrates a typical experiment.

The interrelations between unsustained and self-sustained responses were mutual. Not only did a tonic-clonic response modify the unsustained responses to single shocks but the converse was also true. Facilitation of self-sustained activity by unsustained responses was probably at the basis of all the tonic-clonic responses. Inhibition of some of the components of the tonic-clonic sequence by intermittent single shocks is illustrated in figure 33. Each shock caused a decrease and a slowing of component II.

D. Independence of the Spontaneous Activity and the Different Cortical Responses. The suggestion has been made (Bremer, 1938) that in the records of spontaneous activity and in those corresponding to sensory stimulation and to applications of strychnine the same cortical elements are involved. The different patterns would then depend on the number of active elements and on the degree of synchronization of their discharges. Consistently with this view

Bartley and Bishop (1933) found that in the occipital cortex the amplitude of the responses to electrical stimulation depended on the time of stimulation with regard to the spontaneous cycle—a fact suggestive of the involvement of the same elements in the two modes of activity.

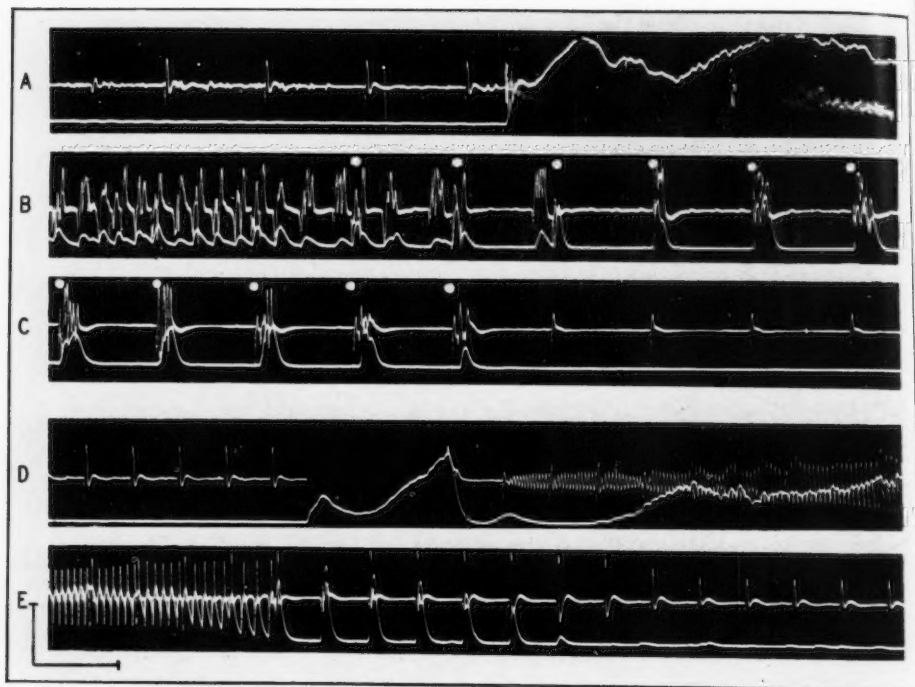


Fig. 32. Motor responses of a left arm muscle to single shock stimulation of the right or left area 4 after a tonic-clonic response. Electrogram (upper tracing) from the primary motor point of the recording muscle on the right area 4 arm. Mechanogram (lower tracing) from the left finger flexors. Voltage calibration: 1 mv.

In A, B and C single shocks were applied at regular intervals to the right area 4 face at the rate shown by the electrogram in A, and by the dots in B and C. The large rise of tension in A corresponds to the period of repetitive stimulation of the left area 4 arm. B shows the end of the tonic-clonic response elicited by the repetitive stimuli. D and E illustrate a similar observation but with the single shocks delivered at regular intervals to the left area 4 arm and the repetitive stimulation applied (during the interruption of the electrogram in D) to the right area 4 face.

Both unsustained and self-sustained responses were frequently found in the present observations to decrease or abolish the spontaneous activity (figs. 16, 17, 30 and 34). This depression is interpreted, however, as indicating inhibition by different elements, rather than refractoriness of the same neurons. Thus, in figure 34, although the spontaneous activity of area 4 arm was decreased during the period of weak stimulation of the ipsilateral area 4 face, this depression is

mainly apparent as a change of rate rather than amplitude. The full-sized spontaneous waves which occurred during that period oppose the view that depression was due to refractoriness. The independence of the elements involved in spontaneous activity before stimulation from those contributing to a self-sustained response is strikingly illustrated in figure 35. The spontaneous activity of area 6 *arm* was promptly depressed upon stimulation of the contralateral symmetrical region, but large rhythmic self-sustained waves of type IV gradually built up during stimulation and persisted after it ceased.

According to the foregoing interpretation, the depression of spontaneous activity which often follows a tonic-clonic response is due to inhibition, not to

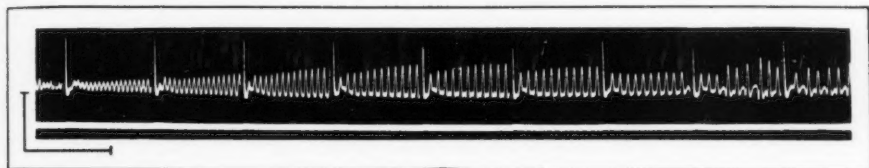


Fig. 33. Inhibition of self-sustained activity in area 4 *arm* by single shock stimulation of the ipsilateral area 4 *face*. The records are as in figure 32. The self-sustained activity was elicited by repetitive stimulation of the left area 4 *arm* with an intensity inadequate for motor response of the recording muscle. Voltage calibration: 1 mv.

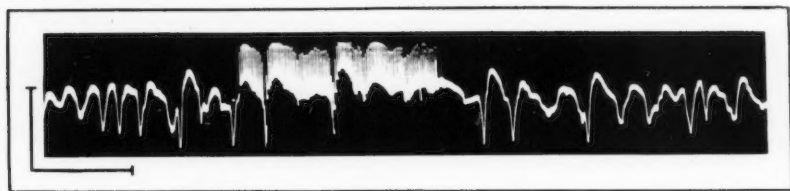


Fig. 34. Inhibition of spontaneous activity of area 4 *arm* upon weak stimulation of the ipsilateral area 4 *face*. Transe cortical record. The repetitive stimuli caused slight movement of the contralateral face, but no arm movement. Voltage calibration: 1 mv.

exhaustion. In support of this view is the fact that regions distant from the stimulated area showed usually no cortical depression following a tonic-clonic response, even when that response was prominent and prolonged (figs. 9 and 10). It may be inferred that, in the spread of a self-sustained response, inhibition does not diffuse as widely or does not endure as long as does excitation.

E. Cortical Responses to Stimulation of Spinal Afferent Nerves. The purpose of these observations was to see whether stimulation of an afferent nerve would cause the appearance of a tonic-clonic cortical response. The procedure followed was to record from area 1 on both hemispheres and to stimulate the sciatic nerve centrally, after peripheral cutting, for different periods with various frequencies and intensities.

The cortical responses to afferent stimulation of the sciatic nerve have been

studied in the cat by Forbes and Morison (1939). They described two components, denoted as primary and secondary responses, respectively. The primary response had a latency of 10 to 12 msec., it was sharply limited to the

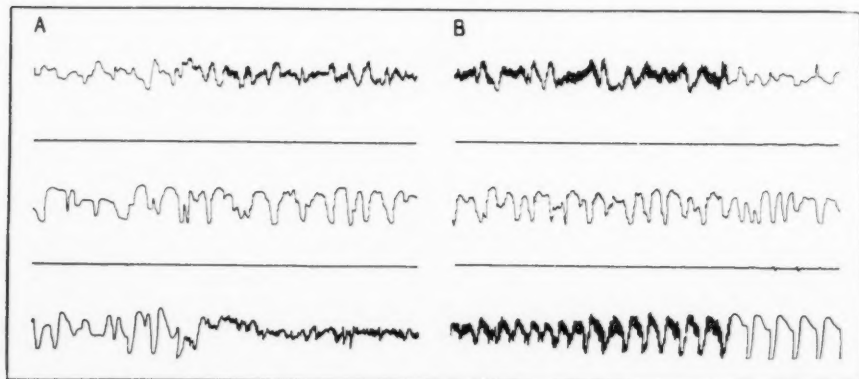


Fig. 35. Inhibition of spontaneous activity of an area, preceding its involvement in a tonic-clonic response (lower record). Moving-coil galvanometer tracings. The records are from above downwards as follows: left area 4 leg, left area 4 arm, and left area 6 arm. A shows the beginning and B the end of a 10-sec. period of repetitive stimulation of the right area 6 arm. The calibrations are as in figure 15.

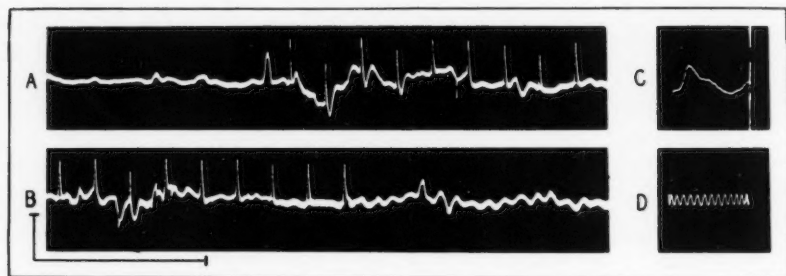


Fig. 36. Responses of the right area 1 leg to central stimulation of the left sciatic nerve at the rate of 5 per sec. Transcortical records. Upward excursions denote positivity of the surface. A and B, continuous film; beginning and end of a 6-sec. period of stimulation. Voltage calibration; 1 mv. C, single sweep to show latency of response to a single shock. D, time calibration for C: 200 cycles.

contralateral sensory area; it could follow rates of stimulation of about 7 per sec. with a decline of amplitude between 20 and 50 per cent of the initial deflection. The secondary response had a latency of 40 to 80 msec., it could be detected over relatively widespread regions in both the ipsilateral and the contralateral hemispheres; it could not follow rates of stimulation greater than about 4 per sec.

Forbes and Morison used deep barbiturate anesthesia; under light narcosis

the cortical spontaneous activity was prominent and the responses to sciatic stimulation minimal or absent. Indeed, the secondary response failed to appear even under deep anesthesia when a stimulus was delivered shortly after a cortical spontaneous wave.

In the present observations cortical responses to sciatic stimulation were seen both with deep and with light anesthesia. The responses differed from those observed by Forbes and Morison in several respects, as follows. Primary responses, i.e., surface-positive responses with a latency of about 8 msec., could be recorded in both the ipsilateral and the contralateral (fig. 36) areas *1 leg*. These responses could follow without significant decline frequencies of stimulation of about 8 per sec. At a rate of 16 per sec. the response was still recognizable, although reduced to about 30 per cent of the original.

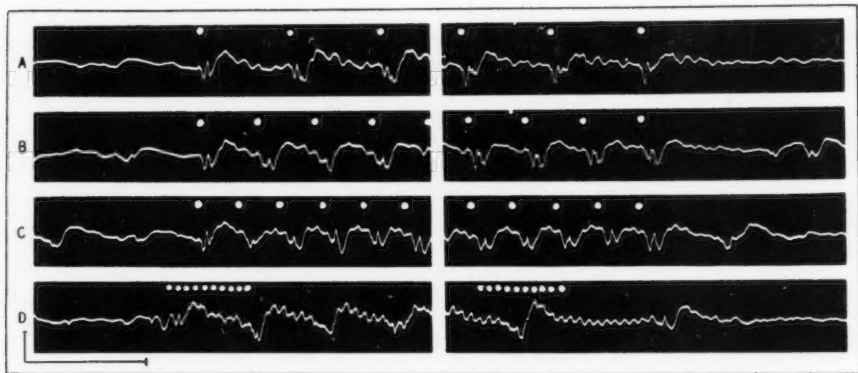


Fig. 37. Cortical responses of area *1 leg* to stimulation of the ipsilateral sciatic nerve at various frequencies. Transcorical records. Upward excursions denote positivity of the surface. Each pair of strips shows the beginning and end of a 10-sec. period of stimulation. The dots indicate the beginning and end of application of the stimuli; in D only 10 shocks at the beginning and 10 at the end of stimulation are signaled. Voltage calibration: 2 mv.

Several components later than that just described were recorded (figs. 37 and 38). At slow frequencies of stimulation the primary effect was followed by a train of damped oscillations similar to component V of the self-sustained activity (cf. figs. 21 and 37). These waves built up with the successive shocks in a repetitive train. Upon increasing the frequency of the stimuli the rate of these waves increased also so that with a frequency of stimulation of 20 per sec. a one to one relation obtained—i.e., there was one wave per stimulus. At higher frequencies of stimulation this ratio failed.

With frequencies of about 5 per sec. or more an additional component appeared in the form of a large slow wave (figs. 37 and 38) which did not follow the rate of stimulation, but which recurred rhythmically with rates of from 2 to 4 per sec. These waves resemble component IV of the self-sustained responses (cf. figs. 20F and 38E).

Cortical discharges which outlasted for several seconds a period of stimulation were often seen (figs. 36, 37 and 38). The components which constituted this residual activity were the damped oscillations and the large slow rhythmic wave described above. On the other hand, typical tonic-clonic sequences could not be elicited, even when high frequencies of maximal stimulation were applied for prolonged periods.

F. Responses of the Striatum, Thalamus and Cerebellum. The purpose of these

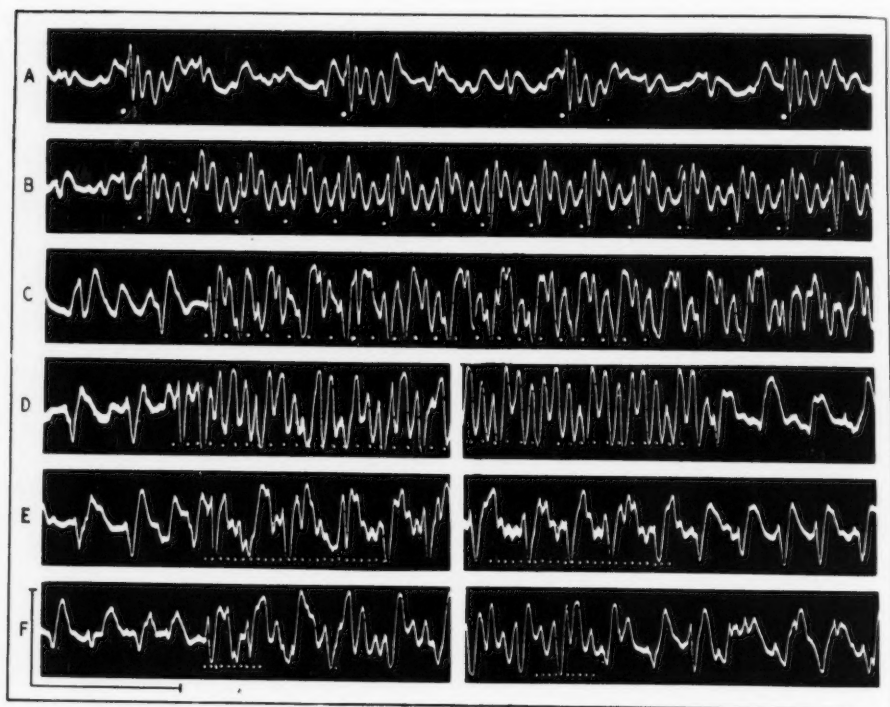


Fig. 38. Cortical responses of area 1 leg to stimulation of the contralateral sciatic nerve. Records and conventions as in figure 37, except that the voltage calibration corresponds to 1 mv.

observations was to see whether self-sustained activity could be elicited in these structures by direct electrical stimulation.

For stimulation and recording of the striatum and thalamus a decortication was performed. The results were entirely negative—i.e., no matter how strong, frequent, or prolonged the stimulation no residual self-sustained activity was seen in these centers.

The observations on the cerebellum were made after excision of one or both occipital lobes of the brain. The tentorium was then removed up to the venous sinuses, thus exposing the upper surface of the lateral cerebellar lobes. As was

true for the striatum and thalamus, direct stimulation of the cerebellum did not result in self-sustained activity. Some of the observations made on this organ, however, are of interest, as follows.

Stimulation of the sciatic nerve causes cerebellar responses (see Dow, 1939), mainly in the ipsilateral, but also in the contralateral side. These responses showed several components, illustrated in figure 39.

While no responses of various areas of the cerebral cortex (ipsilateral and contralateral 9, 8, 6, 4, 1 and 2) were seen from stimulation of the cerebellum, cerebellar responses were readily recorded upon stimulation of the cortical areas 9, 8, 6 and 4 (see Curtis, 1940b). A series of such responses, elicited in the left culmen by stimulation of the right cortical area 4 arm is illustrated in figure 40. As was found for the cortical responses (fig. 29), the cerebellar responses consist of several components (cf. fig. 40F and A). Upon repetitive stimulation these

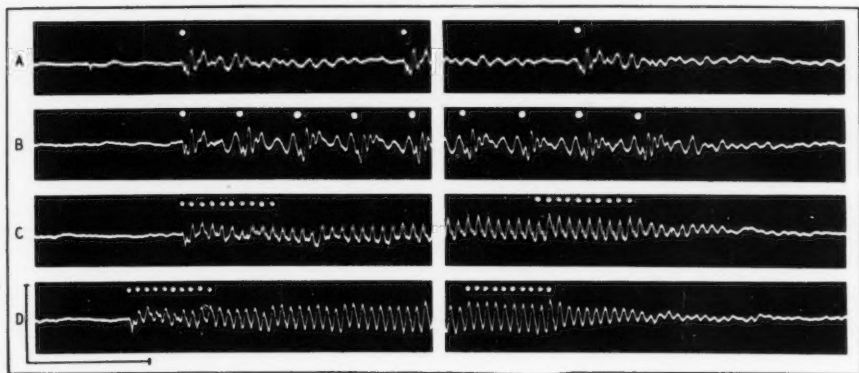


Fig. 39. Cerebellar responses to stimulation of the ipsilateral sciatic nerve. Transcortical record from the left lateral lobe of the cerebellum. Conventions as in figure 37. Voltage calibration: 1 mv.

components vary independently (C, D and E). A decrease of some of the components takes place at relatively high frequencies (C to F). An initial decrease may be followed by a later increase of amplitude during a repetitive train (B, D, E and F). The latency of some components may first increase and then decrease with repetitive stimulation (B to F).

Since the cortex can drive the cerebellum the question arose whether or not self-sustained cortical activity would have a cerebellar concomitant. The question was answered positively by observations in which simultaneous records were taken from the cerebellum and the frontal cortical areas after stimulation of these areas. The cerebellum exhibited responses quite parallel to those recorded from the cerebral cortex.

DISCUSSION. A. *Interpretation of Electrocorticograms.* The expression "components of an electrical record" was used largely as a descriptive aid in the report of the experimental observations. Waves with specific and characteristic amplitude, frequency, form, and sometimes polarity and orientation were

singled out. The question arises whether such specific components represent activity of specific groups of neurons or whether the same neurons can give rise

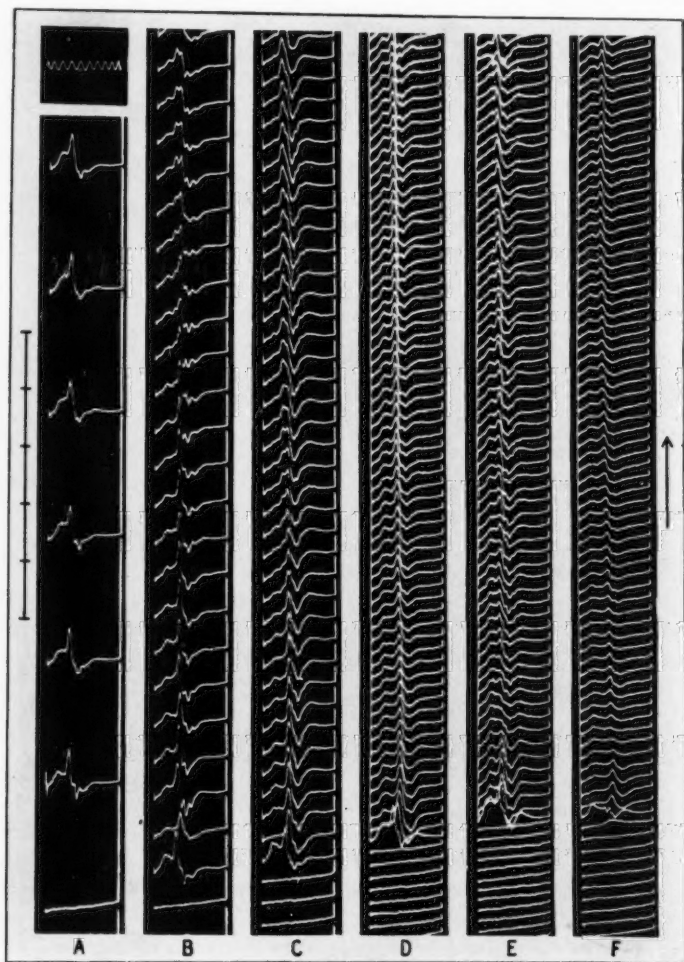


Fig. 40. Cerebellar responses to stimulation of the contralateral cortical area 4 arm. Transcortical records from the left lateral cerebellar lobe. The strips show, from below upwards, the responses to the successive shocks in repetitive series at the following rates: A, 1.3; B, 4.4; C, 7.4; D, 12.0; and E, 13.5 per sec. F was taken at the same frequency as D, but with weaker stimuli than for the rest of the records. Voltage calibration: mv. The time calibration at the top of record A corresponds to 200 cycles.

to electrical phenomena with quite different spatiotemporal characteristics. The evidence favors the view that different components correspond to activity of different elements or organized groups of elements. Were a homogeneous set

of neurons responsible for the several stages seen in a tonic-clonic cortical response a uniform transition would be expected between those stages. But the transition is as a rule abrupt (figs. 9 to 11; section B, *g*).

The same question may be approached from a different standpoint and receives the same answer. The cerebral cortex is a heterogeneous structure. Activity of the different elements would be expected to show the differences which permit the separation of characteristic components in the records.

A corollary of this inference is the conclusion that, since the self-sustained activity follows a similar sequence throughout the cortex (figs. 14 to 16; section B, *c*), different cortical regions have a similar functional organization.

Some of the recorded components differ markedly from the electric responses yielded by the activity of axons. The cortical responses may be much more prolonged than usual axon responses; and they may appear monophasic in conditions in which axon conduction would yield diphasic records. Adrian and Matthews (1934) interpreted the relatively slow cortical waves as due to a summation of temporally dispersed fast components, similar to axon spikes. The data in section C, *a* and *b* (figs. 22 to 25) do not support this interpretation. Slow waves may be recorded at a point in the cortex by stimulation only 1 or 2 mm. away—i.e., in conditions which should minimize temporal dispersion.

It appears more likely, therefore, that some of the electric phenomena recorded from the cortex are not of "axon," but of "cell" origin (see Bartley and Bishop, 1933; Bremer, 1938). Cell potentials might differ from axon potentials only quantitatively; that difference would be relatively unimportant. The significant question is whether excitation and conduction in synapses, dendrites and cell-bodies differ qualitatively from the corresponding processes in axons. The following considerations support the existence of qualitative differences.

In the experiments illustrated in figure 24B the polarity of some of the components in the responses was not reversed by stimulation first on one, then on the other side of the recording leads. Axon-like conduction would have resulted in a reversal of polarity in these conditions.

In his study of the spread of activity in the cerebral cortex, Adrian (1936) recognized two types of response; in one the lead at the surface of the active region went negative, in the second it went positive, with respect to another distant lead. The first type was interpreted as denoting responses of superficial elements, hence the negativity of the surface; the second, as denoting activity of deep elements, hence the relative superficial positivity. The applicability of this interpretation to the present observations was controlled in the experiments illustrated in figure 28. If positivity at the surface invariably denoted negativity at deeper layers, then introduction of the electrodes till they come in contact with these deep layers should result in a reversal of sign of the response. This reversal did not take place (fig. 28).

The following premises have been adopted for the interpretation of cortical potentials. A difference of potential between two cortical regions corresponds to asymmetric changes in some of the elements included between the leads. The response will be large if there are many such elements with a parallel orientation. For a given response the position of the leads with respect to the

field will determine the amplitude of the deflections recorded. Relative positivity of some region *a* at the surface with respect to another surface lead *b* need not be due to deep negativity at *a*, but may correspond to any changes in the elements between the two leads that set up a field of the proper polarity. As in

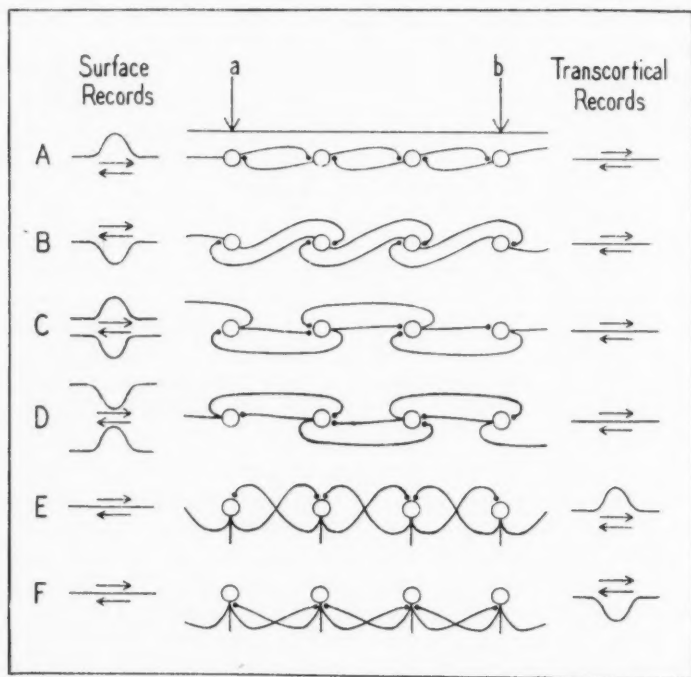


Fig. 41. Diagrammatic representation of possible neuron chains which would yield electric responses of various orientations and polarities. In the middle drawings are represented cells arranged parallel to the surface. It is assumed that each element activates the following element. It is also assumed that when such activation takes place the region of the cell body in which the synaptic connection with the previous element is placed becomes enduringly electronegative with respect to the rest of the cell.

Stimuli are supposed to be applied beyond each of the recording surface electrodes *a* and *b*. The arrows point the direction of spread of the wave. In the left diagrams are shown the responses which would occur with each of the neuron connections shown; upward excursions correspond to negativity of the lead proximal to the stimulated point. In the right diagrams are shown the corresponding transcortical records; upward excursions correspond to negativity of the surface with respect to the deep regions.

smooth muscle (Rosenblueth, Davis and Rempel, 1936), the potentials corresponding to the elements included between the leads sum in series, hence the larger responses with greater interelectrode distance (fig. 22B).

The diagrams in figure 41 illustrate these premises. The following assumptions are made. Each cell is capable of activating the neighboring elements;

the corresponding synaptic connections are represented by knobs. The region of the cell body where the synaptic connection lies becomes negative with respect to the rest of the cell for a period relatively long (compared to the propagation velocity) during the process of activation; this assumption provides the asymmetry indispensable for the external detection of a potential drop. The alternative assumption, that the region of activation becomes relatively positive instead of negative, would be no more arbitrary than that adopted, for there is no evidence at present bearing on the problem.

With these assumptions a propagated wave would be recorded by surface electrodes as follows (left diagrammatic records, upward excursions denote negativity of the lead proximal to the stimulating electrodes). In A the lead proximal to the stimulated region (beyond the *a* or *b* recording electrodes) would be negative with respect to the distal lead; in B the reverse would occur. In C lead *a* would be negative with respect to *b*, regardless of the point stimulated; in D the reverse would occur. In A to D the transcortical records (right diagrammatic records) would show little or no deflection. In E and F the transcortical records would be maximal, while the surface leads would be practically isopotential; in E the surface would be negative with respect to deeper layers; in F the reverse would occur. A summation in series of the potential drops would take place in A to D, but not in E and F.

All of the cases schematized in the records in the diagram were encountered in the observations. Thus, components which recorded superficially but not transcortically (diagrams A to D) may be seen in figure 27; and components whose polarity depended on the point stimulated (diagrams C and D), and also components with a polarity independent of the site of application of the stimuli (diagrams A and B), are illustrated in figure 24B.

B. *Correlation between electrocorticograms and muscular activity.* As shown in figure 11, it is not possible to determine by observation of the electrocorticograms alone whether simultaneous muscular activity is present, and the degree of this muscular activity. The lack of correlation between the two records is probably attributable to several factors. Thus, elements other than the projection motor cells contribute in all probability to the cortical record. It is not feasible, therefore, to determine the share of these projection elements in the total response. This statement is in agreement with Bremer's (1938) observations and with Adrian and Moruzzi's (1939) conclusion that the potential waves in the motor area and the discharge in the pyramidal tract are closely related but are not inseparable.

Even if the efferent impulses, pyramidal and extrapyramidal, were recorded, an absence of correlation between these discharges and motor activity could take place (cf. Adrian and Moruzzi, *loc. cit.*). Efferent impulses from the cortex do not invariably lead to stimulation of motoneurons. Inhibition of spinal reflexes can be readily elicited by cortical stimulation (Rioch and Rosenblueth, 1935).

The contribution, excitatory or inhibitory, from subcortical centers to a given motor response, cannot be appreciated from a corticogram. That other centers

than the cortex and the spinal cord are probably involved in motor responses is shown by the observations made on the cerebellum (p. 726).

The absence of correlation between the cortical records and the motor effects has been labored because it throws some light on the limitations of electroencephalographic observations. The suggestion emerges that the observations of cortical potentials will illuminate a given physiological process in direct relation to the degree of corticalization of this process and to its simplicity—i.e., a purely excitatory or a purely inhibitory process as opposed to one in which there is a mixture of excitatory and inhibitory components.

C. *The Self-Sustained Cortical Responses.* a. *Degree of response.* The intensity and frequency of the stimuli and also the length of time that they are applied decide whether self-sustained activity will ensue and the amplitude and degree of spread of that activity (sections A, c and B, c). With any 2 of those 3 parameters of the stimuli constant (see Dusser de Barenne and McCulloch, 1939), variation of the 3rd one will reveal a threshold below which no self-sustained activity is elicited, and above which there is a direct proportionality between the response and the degree of stimulation.

Increase of intensity of the shocks results in the stimulation of a greater number of cortical elements per shock (spatial variation). Increase of frequency produces temporal variation of a given number of elements. Since such changes determine the appearance and the magnitude of the responses it may be inferred that temporal and spatial effects are largely interchangeable with regard to self-sustained activity. Variations of the period of stimulation are also of significance for the tonic-clonic responses. This influence cannot be explained reasonably on the basis of the activity of the elements stimulated directly, but it suggests that temporal summation in elements activated indirectly is one of the determining factors for the appearance of a response.

The influence of the 3 parameters of the stimuli may be summarized by the statement that the responses are determined by the total "quantity" of stimulation delivered to the cortex.

b. *Components of the response.* Once the threshold quantity of stimulation has been reached the self-sustained activity begins and it begins by the fast waves designated as component I (fig. 20A). This stage may or may not be followed by the subsequent components. Indeed, a response may consist only of I, or of I followed by II, or finally it may show the complete sequence described in section B, a. These differences are readily explained by the interpretation that the several components correspond to activity of different groups of elements (p. 728). The cells which discharge during component I would activate those that yield component II, and these in turn would bring into play the elements which give components III and IV during the clonic bursts. If each subsequent set of elements requires a critical degree of excitation for its independent activity the response may stop at any component, when the threshold for the next set was not reached.

The interpretation that self-sustained responses consist of the successive induction of activity of different groups of elements and that each of these groups

has a fixed threshold of quantity of stimulation, differs from other previous interpretations. Thus, Bremer (see Moruzzi, 1939) suggests that the self-sustained responses are merely the prolongation of the synchronizing action of cortical activity caused by the stimuli applied; any faradic stimulation, even weak, would synchronize the cortical elements and would effectively result in "experimental epilepsy." According to this suggestion there is no "neural" threshold for the responses, only the electrical threshold of the cortical cells. The maximum of the response should then occur at the time of stimulation. This suggestion is contradicted by the fact that there is a threshold quantity of stimulation (p. 732) and that the responses need not be maximal at the time of stimulation, but may build up, even at the stimulated region, for some time after the stimuli have stopped (figs. 9 to 11).

The possibility that the progressive slowing of the discharges in a response may correspond to a process of synchronization of the elements discharging in a given area should be considered. If this were the case the amplitude of the waves should increase when the frequency slows, and decrease when the frequency is higher. In some of the observations described on p. 709 amplitude-time graphs were drawn in addition to the frequency-time graphs. Although in general there is an increase of amplitude as the frequency decreases this correlation is only a broad one. It is true that the frequency decreases as the response shifts from component I to II and then to III and IV, while the amplitude of these components increases from I to III. But within the periods occupied by any of these components the frequency of discharge may increase or decrease markedly without any reciprocally correlated change in amplitude. Similarly, the change of frequency illustrated in figure 1B was not attended by any corresponding change of the amplitude of the muscular clonic contractions.

c. *Rhythmic activity.* Self-sustained repetitive discharges may be explained on the basis of reverberating circuits (Ranson and Hinsey, 1930) or may be attributed to the intrinsic ability of some elements to discharge rhythmically in the absence of impinging nerve impulses (Adrian, 1936). Several arguments make the first hypothesis unlikely.

If reverberating circuits were responsible for the enduring tonic-clonic discharges the rate of the responses would be mainly a function of the length of the closed paths. Elaborate sub-assumptions are necessary, therefore, in order to account for the changes of frequency during a response and for the very slow clonic rates. According to the hypothesis the duration of a response should depend mainly on the properties of the reverberating neuron chains, rather than on the characteristics of the stimuli. The dependence of the activity on the quantity of stimulation, therefore, does not favor the hypothesis. The reverberating circuits could be either small and localized or they could extend over large regions of the cortex. Short localized chains do not account for the simultaneous end of a response in areas long active and in other distant regions to which the effects may have spread only late after stimulation (fig. 16). Long chains, on the other hand, do not explain why isolation of a relatively small region of the cortex by section (p. 703) does not modify the rate of clonus. There

are anatomical provisions in the cortex for reverberation (Lorente de Nó, 1938) but the functions of such circuits may not be inferred from their anatomy. Thus, in the cerebellum there are also anatomical circuits which could lead to reverberation, yet electrical stimulation of the cerebellum does not result in self-sustained activity (p. 726).

It is more probable, therefore, that rhythmic discharges during self-sustained responses are due to the intrinsic ability of some cortical cells to fire repetitively during and after a period of excitation.

d. *Spread of activity.* The obvious suggestion for the spread of self-sustained activity is that the discharging neurons of the stimulated area are the main source of activity and the pace-maker of a response. Several objections may be raised against the unrestricted acceptance of this suggestion. As pointed out before (p. 707), the data do not support the existence of a localized pace-maker. If the stimulated area were the main source of the tonic-clonic activity, then removal of this area during a response which has spread to other regions would result in a prompt cessation of activity. This is not the case (p. 707; see Bubnoff and Heidenhain, 1881; François-Franck and Pitres, 1883).

The stimulated area starts the responses, but once other areas are active they probably become in turn sources of impulses both to the stimulated and to other regions. This view explains the previous difficulties but leads to the inference that self-sustained activity should always spread like a landslide until complete generalization. Since this is not the case it is necessary to conclude that there are factors which tend to suppress self-sustained activity. These factors will be discussed below (p. 735).

According to Adrian (1936) spread of activity occurs from cell to cell by lateral connections of the pyramidal elements. Each additional element only begins to discharge rhythmically after repetitive facilitation. This explanation seems unlikely. There is no evidence that the agents for spread are pyramidal cells. Certainly when crossed responses appear the activation of new elements is not produced by side to side propagation, but by long pathways making synaptic connections (Erickson, 1940). Even for spread within one hemisphere, the possibility of obtaining cortical self-sustained activity in area 4, without muscular contractions (fig. 11A and B), is opposed to the concept that propagation is carried out by pyramidal cells. Adrian's suggestion is applicable to propagation of some of the components of unsustained responses but is improbable for spread of self-sustained activity.

Spread of tonic-clonic activity is probably due to persistent synaptic bombardment of some elements by similar active elements. New elements come into play only after the excitation caused by this bombardment has summed over relatively long periods of time, hence the slow propagation. Under chloralose anesthesia, spread within one hemisphere is mainly to points in the neighborhood of the active area or areas—the pathways over which the impulses travel are probably the short connections which exist practically everywhere in the cortex. Long pathways are demonstrated by the crossed effects. It is not

necessary to assume that other elements than those which yield component I are involved in the spread of responses, since activity of any area always starts with this component.

e. *Synchronism.* The early stages of a self-sustained response are not synchronized in different areas. Each of these areas may discharge with its own independent rate during the periods corresponding to components I and II. Early during the clonic stage, however, i.e., when components III and IV become organized as clonic bursts, all active areas discharge at the same rate and almost simultaneously (figs. 14 to 16).

The suggestion has been made by Bremer (1941) that synchronization of activity in the strychninized spinal cord may be caused by a purely electrical non-synaptic intercellular influence. This suggestion does not apply to the coupling of activity in distant cortical areas during clonus. The coupling is similar for areas in one hemisphere and for contralateral areas, and the crossed coupling can only be produced by synaptic influences (Erickson, 1940). Thus, the synchronism of the clonic bursts reveals a system of nerve pathways interconnecting some elements in all cortical areas.

f. *The end of a response.* The abrupt cessation of clonic activity in all the regions sharing in a response is a dramatic and puzzling phenomenon. The hypothesis has been often advanced that the end of a tonic-clonic response is due to cortical exhaustion. As pointed out by Adrian (1936) this hypothesis does not explain satisfactorily why activity ceases simultaneously in the region where the response was initiated, and in regions to which the response spreads only after some time, and which, therefore, discharged only for a relatively short period. Furthermore, a test for exhaustion by stimulation immediately at the end of a response can reveal a state of facilitation (figs. 31 and 32) instead of the deep depression which would be expected.

Three alternative hypotheses may be considered: inhibition (Bubnoff and Heidenhain, 1881), the accumulation of metabolites (see Adrian, 1936; Dusser de Barenne and McCulloch, 1939), and a relative refractoriness of the responding elements. The same arguments which invalidate the hypothesis of exhaustion render these three alternative views unlikely.

The following explanation accounts satisfactorily for the data. In the course of a tonic-clonic response there is a gradual slowing of the frequency of intrinsic rhythmic discharge of the several elements which contribute to the response (p. 709). This slowing is probably not due to increasing refractoriness, for it is possible to interpose responses between the clonic bursts that do not differ significantly from these clonic bursts (fig. 31). The slowing may be due to gradual waning of the excitatory process which initially changed inactive to rhythmically active elements.

It is assumed that persistent spontaneous rhythmic discharges cannot occur below a critical degree of excitation, and hence, below a critical frequency which may vary with the experimental conditions. In other words, it is assumed that a minimal frequency is necessary for temporal summation adequate for continu-

ous rhythmic discharge. In the present experiments, in all animals and in all areas the responses ceased as soon as the frequency of clonic discharge slowed to 1.0 to 1.5 per sec.

According to this explanation the response stops because excitation has waned, not because the responding elements are depressed by previous activity. The difficulties encountered for the acceptance of the other hypotheses mentioned are thus obviated. The explanation accounts for the fact that self-sustained responses do not invariably generalize (see p. 734). As new areas join a response, their rate soon slows to that of the other active regions (fig. 16). If the degree of stimulation is small the response in the initially active region will be brief—i.e., it will slow promptly. The response will then end before it has had time to spread to many areas. This explanation is compatible with the view that the stimulated region is not the pace-maker of the response (see p. 734). The extent of spread, on the other hand, is made, within limits, a function of the amount of excitation developed at the stimulated region.

Under dial anesthesia (observations on the 1 animal experimented on with W. S. McCulloch, and personal communication from McCulloch) and very rarely under chloralose (2 out of 18 animals in which multiple recording was made) a response may end in one area while it still endures in another region. These animals might be considered to invalidate the explanation adopted. It should be mentioned, however, that the suggested hypothesis does not exclude the probable rôle of other factors, especially of inhibition. Furthermore, if the physiological coupling (p. 735) between different areas were loose in some animals or in some experimental conditions, a relative independence of those different areas would ensue.

D. The Tonic-Clonic Experimental Responses and Clinical Epilepsy. The purpose of this section of the discussion is not to explain clinical epilepsy but to point out suggestions from the experimental results that have a bearing on the clinical problem.

Phenomenologically, the muscular reactions during a tonic-clonic response to electrical stimulation of the motor cortex have for a long time been recognized as analogous to those in Jacksonian or in grand-mal epilepsy—hence the term experimental epilepsy. If the analogy is significant, i.e., if the physiological process is similar in the two cases, then the experimental findings strongly support Jackson's (1890) and Wilson's (1929) views that the radiation of an epileptiform fit is entirely a physiological process and that most or all of the cortical regions involved in an epileptic fit may be normal.

Jackson's and Wilson's idea, that the fit is produced by a sudden, excessive, temporary liberation of energy in some motor nerve cells, need only be modified to read "a sudden marked degree of stimulation of a cortical region or an increased sensitivity in some cells" to reconcile it with the experimental data.

The findings that tonic-clonic activity may occur in any cortical region (figs. 14 to 16) and that it may spread to the motor area from quite distant cortical points (p. 695) provide a physiological basis for the interpretation of auras and of epileptic variants.

If the epileptiform reaction is interpreted as a physiological process the question arises, why do epileptic attacks occur only in certain subjects? The answer is obviously that normally the degree of excitation of the cortex does not reach at any point the threshold for pronounced tonic-clonic cortical activity. Two possibilities would then explain the appearance of fits in some subjects.

First, it is probable that there are limitations to the number of afferent impulses which can reach the cortex in a given time. Thus, when the sciatic nerve is stimulated at high frequencies the cortical responses do not follow the stimuli (figs. 37 and 38). Morison and Dempsey (in press) have recorded one-to-one cortical responses from stimulation of the thalamus up to 120 per sec. It is conceivable, therefore, that if the "filtering" action of the thalamus were impaired in some pathological condition the cortex could receive more stimulation than normally. Such a mechanism could account for some of the "reflex" epilepsies.

The second possibility is that in pathological conditions some region of the cortex acquires a lower threshold than normal for self-sustained responses (see Penfield and Keith, 1940; Obrador, 1941). A normal degree of stimulation could then start rhythmic self-sustained discharges from that region which would propagate in the normal manner. If this were the condition of a patient the suggestion emerges that local extirpation of the abnormal area, diagnosed by the aura, might eliminate the fits, much as local extirpations can eliminate Jacksonian fits. The experimental data suggest that the differences between epileptic variants are merely differences of localization and quantitative differences (see Jasper and Kershman, 1941).

E. Some Properties of the Cerebral Cortex. a. *Different types of cortical responses.* Throughout this report the cortical responses are divided into three groups: direct, indirect unsustained, and self-sustained. This systematization needs no argument, since it is more descriptive than explanatory. The distinction between self-sustained and unsustained responses should, as a rule, be easy. The terms direct and indirect, on the other hand, are relative to the experimental conditions. Thus, the same elements may respond directly to stimulation, or indirectly to nerve impulses set up by stimulation elsewhere.

In physiological conditions the cortex is never stimulated directly. Yet the distinction may be useful between the responses set up by afferent nerve impulses at the primary sensory area and the secondary responses elicited by the activity of primary elements.

Some of the "spontaneous" discharges usually recorded from any cortical region are probably self-sustained—i.e., they do not require continuous afferent bombardment of impulses of non-cortical origin, since the isolated occipital pole (p. 703) exhibits some spontaneous waves. The self-sustained responses differ from the spontaneous activity in several respects. The responses take place only after relatively abundant stimulation. They do not appear in the records as an intensification of the spontaneous background, but as an independent phenomenon. Indeed, the responses are usually preceded and attended by inhibition of spontaneous activity (figs. 16 and 35).

It is likely that some cortical elements may share in several types of activity. Thus, the inhibition or facilitation of unsustained responses during the development of self-sustained discharges (figs. 31 and 32) suggests that some cells are common to both reactions. And the pyramidal cells are an efferent pathway common to many motor reactions of cortical origin. It is also likely, however, that specific responses involve only some specific elements in any cortical region.

The systematization adopted for the cortical responses has a bearing on the problem of the delimitation of the motor areas of the cortex. While the early workers in the last century mapped very extensive regions of the cortex from which movement could be obtained, the tendency in more recent studies has been to narrow the extent of the motor area and to attribute some of the early results to spread of the electrical stimuli. Many studies have shown that the efferent motor pathways from the cortex (pyramidal and extrapyramidal) arise from limited cortical regions—mainly areas 4 and 6, and also from 8, 19 and 22 (for references see Fulton, 1938). Only from these areas may direct motor responses be elicited. Indirect responses, on the other hand, both unsustained and self-sustained, may occur as the result of stimulation of practically any cortical region, according to the present observations. The "spread" in the early studies may thus have been not spread of the electrical stimuli, but spread of cortical activity.

b. *Spread of cortical activity.* Both the unsustained and the self-sustained responses tend to spread both in the ipsilateral (see Adrian, 1936) and in the contralateral hemisphere (Erickson, 1940). The spread of self-sustained responses was discussed on p. 734. The waves of unsustained discharge do not require temporal summation, since the responses are obtained by single shock stimulation. The influence of spatial reinforcement is shown by the greater extent of propagation of the responses to strong than to weak shocks. Temporal facilitation becomes obvious when some components increase and travel further upon repetitive stimulation at adequate rates (fig. 29).

Both the unsustained and the sustained responses decrease in amplitude as the distance from the region stimulated increases (figs. 22A and 25; 14 and 15). Thus, spread of activity in the cortex may be spoken of as decremental. The decrement of responses with distance may be explained by a progressive reduction of the density of synaptic connections—i.e., by a gradual diminution of spatial facilitation.

As already mentioned (p. 706), Adrian's (1936) study and the present observations emphasize the existence in the cortex of mechanisms for relatively indiscriminate spread of activity. That there are, however, preferential connections between certain areas was brought out by the work of Dusser de Barenne and McCulloch (1938) and that of Moruzzi (1939).

c. *Tendency for synchronization.* Only one further comment need be added to the discussion on p. 735. There is a tendency for synchronization of nervous activity in many experimental conditions (see Adrian and Matthews, 1934). There may be, however, different mechanisms which lead to this synchronization

in different observations. Thus, synchronization of the discharges of injured axons is obviously due to non-synaptic factors. Similarly, Bremer's (1941) study of the action of strychnine on the spinal cord suggests again that the factor for synchronization may be non-synaptic. The synchronization of clonic activity in widely distant areas in both hemispheres, on the other hand, is probably due to synaptic coupling of the responding elements (p. 735).

d. *Facilitation and inhibition.* An extensive discussion of facilitation and inhibition in the cerebral cortex would be out of place here. The present data, however, have a bearing on some aspects of these problems.

It has been suggested by Dusser de Barenne and McCulloch (1939) and by Moruzzi (1939) that increased spontaneous activity in a cortical area causes facilitation of the responses of that region, and conversely, that decreased spontaneous activity will depress the reactivity of the area to various modes of stimulation. Moruzzi extends the influence of local discharges on facilitation to cover all modes of activity, whether spontaneous or induced by electrical stimulation. This extension is a corollary of his view that "epileptiform" responses are merely an exaggeration of spontaneous activity. As previously shown (p. 735), this view is not supported by the present data.

As a general statement the concept is justifiable that increased local activity is attended by facilitation of responses and that decreased activity corresponds to local depression. There are, however, signal exceptions to this rule. Thus, the period of great facilitation of both cortical and motor responses which follows the end of a tonic-clonic response (figs. 31 and 32) coincides usually with relative or total cortical silence. Conversely complete abolition of cortical and motor responses may take place during the period occupied by component II in a cortical self-sustained response (p. 721).

The facilitation at the end of a clonic response may be interpreted by further elaboration of the theory outlined broadly on p. 735. Let it be assumed that rhythmic discharges occur when sufficient impinging nerve impulses have raised the level of "excitation" of some elements. Let it be further assumed that this level of excitation progressively declines, together with the frequency of discharge. The response would then stop when the degree of excitation would be less than a critical value (cf. the critical frequency postulated on p. 735), but this value could be higher than the normal resting one—hence the condition of facilitation. These assumptions imply the existence of an enduring excitatory agent, capable of attaining supraliminal levels, a view which has been many times proposed in the past. Such an agent appears necessary to account for rhythmic discharge if, as is probable (p. 733), this discharge is not due to reverberation.

The interpretation of cortical responses is made throughout the discussion with emphasis exclusively on excitatory phenomena. This was done with the deliberate purpose of simplifying somewhat the complicated problems considered. It is very likely that inhibition plays likewise an almost constant part in the effects registered, as was suggested in 1881 by Bubnoff and Heiden-

hain. Many instances of inhibition were encountered in the observations (pp. 696, 720). To attempt to interpret them at present would be premature. The understanding of excitatory and inhibitory interrelations in the cortex awaits further, more elaborate experiments than were made here.

The emphasis in this study has been placed on features of cortical function which are common to all cortical areas. Specific structure of different regions, which is the basis of the classification of the cortex into areas, suggests that some functions of these areas will be found specific, not only because of characteristic afferent and efferent connections but also because of a characteristic local organization. Comparison of the present with previous observations suggests that chloralose anesthesia tends to erase some of the specific differences. This anesthetic is useful, therefore, for the analysis of the properties of the cortex *qua* cortex. These general properties were the main interest of this study.

SUMMARY

The motor and the electric cortical responses to single and to repetitive cortical stimulation were studied chiefly in monkeys and also in dogs and cats. The table of contents at the beginning of the paper shows the observations made and the topics discussed.

We are grateful to Dr. R. S. Morison for his help in the observations on the hippocampus, thalamus and striatum.

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THE CALORIGENIC EFFECT PRODUCED BY VARIOUS MIXTURES OF FOODSTUFFS

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In a recent paper, Ring (1940) showed that the calorogenic action of fat is reduced by the injection of large doses of cortical extract. In that paper it was suggested that the extract brought about storage of glycogen in the liver. This depressed the calorogenic effect by reducing the amount of fat processed in the liver. Under these circumstances, the fat was thought to be stored in approximately the form in which it was ingested. When liver glycogen was low or absent, fat was needed as a source of energy and was modified so as to be suitable for use in the catabolic processes. This transformation is undoubtedly one in which a considerable amount of energy is wasted and may well account for the calorogenic effect.

At the time the above suggestion was made, there was no evidence that the specific dynamic effect of fat was smaller when there were considerable amounts of carbohydrate in the body. In fact the work of Murlin, Burton and Barrows (1936) showed that the calorogenic effects of fat and carbohydrate were additive (see also Murlin and Lusk, 1915). However, in their experiments the carbohydrate was ingested some time after the fat and so was less likely to depress the S.D.A. of fat than if given earlier. In 1939, Forbes, Bratzler, Thacker and Marcy showed that the calorogenic effect of all three foodstuffs combined is less than the sum of the individual calorogenic effects; but they gave no figures for the specific dynamic effect when sugar and fat alone were eaten. We have obtained such figures and also those for protein plus carbohydrate and protein plus fat. The results indicate that the ingestion of carbohydrate plus fat or protein prevents the full calorogenic effect of the individual constituents from appearing. On the other hand, the ingestion of protein together with fat produces a metabolic stimulation which is nearly equal to the sum of the individual calorogenic effects. Each of these latter foodstuffs probably has to be modified in order to be available as a source of energy, whereas carbohydrate needs little transformation and is the fuel of choice.

METHOD. Rats were chosen for all of the experiments described in this paper. The apparatus used to measure metabolism was the one previously described (see Ring, 1940). The rats were all 18 hours post-absorptive at the beginning of the measurements. The oxygen consumption was recorded during the three hours before the test meal was given and for eight hours thereafter. From the graphs, oxygen which was used during part of each hour when the rat was quiet

has been measured. In about one-quarter of the experiments, the respiratory quotients were determined by the Haldane principle. The rats were used only once a week so that they continued to gain weight in spite of the periodic fasts.

In studying the S.D.A. of protein, 3 cc. of 50 per cent Bactopeptone (Difco), a derived protein, were used; for carbohydrate, 3 cc. of 50 per cent glucose; and for fat, 1.5 cc. of oleic acid. First, a single foodstuff was given to each rat, then a mixture, and then the single foodstuff again, etc. By alternating the observations in this way and obtaining at least three results for each foodstuff or mixture, possible errors resulting from changes in age or weight of the animals were eliminated. Occasionally the amount of material given produced diarrhea, and that day's experiment had to be omitted from the calculations.

RESULTS. In table 1 is shown the average increase in oxygen consumption on

TABLE 1

Average percentage increase in oxygen consumption during eight hours following ingestion of food

RAT NO.	GLUCOSE	FAT	FAT AND GLUCOSE	PROTEIN	PROTEIN AND GLUCOSE	PROTEIN	FAT AND PROTEIN
1		10.1	8.5				
2		7.4	2.3				
3		7.3	3.2				
4		10.8	7.6				
5		8.6	0.6				
6		8.1	7.1				
7	5.5			17.3	13.0	17.3	20.1
8	4.4			14.4	13.3	14.4	20.5
9				14.3	10.1	14.3	15.3
10	3.2			9.7	7.1		
11				10.8	8.5	10.8	22.7
12						13.9	15.0
13						11.5	22.8
14	0.9			5.5	0.0	5.5	10.2
Average.....	3.5	8.7	4.9	12.0	8.7	12.5	18.1

three different days during the eight hours after the food was ingested. It will be seen that for each animal the increase in oxygen consumption was larger when oleic acid was given alone than when it was mixed with glucose. Since the caloric value of oxygen varies with the type of food burned, it does not necessarily follow from this that the energy expenditure was less when sugar was given in addition to fat. The calorigenic value of the oxygen consumed can, however, be calculated by using the respiratory quotients given in table 2. It will then be found that the figures representing the increase in energy expenditure when fat is fed will be somewhat smaller than those for oxygen consumed which are given in the second column. The figures for the extra energy expenditure after supplying fat plus sugar will be larger than those in the third column. The difference between the S.D.A. of fat and of fat plus sugar will be correctly shown in each animal if 2 per cent is added to the third column (the figures for fat plus

sugar). In four of the six rats used the calorigenic effect of fat plus sugar was less than that of fat alone. In the other two animals the specific dynamic effect of fat plus sugar though larger than fat alone was much lower than would be expected if the calorigenic effects of the individual foodstuffs were additive. It is, therefore, apparent that when fat and sugar are mixed there is a reduction in the S.D.A. of one or both. The respiratory quotient found in the experiments using fat plus sugar indicates that the S.D.A. of fat is reduced. If carbohydrate and fat shared in proportion to their calorigenic effects in modifying the respiratory quotient, then this should have been 0.750 whereas it was 0.784. Since it is unlikely that carbohydrate catabolism was increased when the mixture was given, the quotient of 0.784 probably indicates that the fat catabolism, superimposed upon the basal metabolism, was lower by 86 per cent after the ingestion of the mixture than when given alone. The S.D.A. of sugar appears to have been normal or at least not reduced by the presence of fat. If the average S.D.A. of sugar is subtracted from the S.D.A. of the mixture, the difference should represent the S.D.A. caused by fat. This figure suggests that the calorigenic

TABLE 2
Average respiratory quotients during eight hours after ingestion of food

Control.....	0.723
Glucose.....	0.796
Oleic acid.....	0.707
Peptone.....	0.737
Oleic acid and glucose.....	0.784
Peptone and glucose.....	0.804
Peptone and fat.....	0.724

effect of fat has been reduced by 86 per cent—the same figure as that found using respiratory quotients as the basis for calculation.

In a series of seven partially depancreatized rats which are not included in table 1, the calorigenic effect of fat plus sugar was found to be much smaller than that of fat alone just as in the normal animals. The oxygen consumption during the eight hours after the ingestion of fat showed an average increase of 12.5 per cent and after fat plus sugar only 6.7 per cent.

If we now consider the calorigenic effects of protein and sugar, it is clear that the oxygen consumption after the ingestion of the mixture is less than that after protein alone (see table 1). In this case, the *calorigenic* effect cannot be accurately calculated unless the excretion of nitrogen or sulphur is known. It can be shown, however, that the calorigenic effect of the mixture is not as great as that produced by these foodstuffs given separately. If we select the lowest possible caloric value for oxygen when peptone was given (this would be the figure for pure protein catabolism) and the highest figure for the experiments using the mixture (in this case one must assume no protein catabolism), then the S.D.A. of the mixture still falls 2 per cent below the sum of the individual S.D.A.s. This is the minimal difference which can be obtained from these

figures. If we make the more reasonable assumption that the breakdown of protein is the same in the two cases, then the calorogenic effect when sugar was supplied with protein gives a figure 5.8 per cent below that obtained by adding the S.D.A. of sugar and protein. The respiratory quotients, as in the case of the previous mixture, suggest that the catabolism of sugar is not reduced when protein is added. If this is correct, then the S.D.A. of protein must have been reduced 59 per cent using the second calculation described above or 27 per cent using the first. The true change in the calorogenic effect of protein lies somewhere between these two figures, probably close to 59 per cent of normal value.

The possibility still existed that the sugar might delay but not reduce the S.D.A. of fat or protein. The observations make it quite apparent that the S.D.A. is not completely over at the end of eight hours in most experiments. However, when the measurements were stopped, the oxygen consumption was closer to the control figures when sugar plus fat were used than when fat alone was supplied. The respiratory quotients was also almost down to the control figures. These facts suggest that the part of the S.D.A. not measured was smaller in the experiments using fat plus sugar than in those in which fat alone was used. When measurements were continued for more than eight hours after the ingestion of food, the rats became increasingly restless and periods of quiet were seldom long enough to be reliable. The experiments using protein offer no better support to the suggestion that the S.D.A. is delayed by sugar.

Another point to be considered is whether mixing the fat with the sugar solution would reduce the S.D.A. because of diluting the fat. In five experiments where a 1 per cent salt solution was mixed with the fat, the S.D.A. of the fat was not reduced. Furthermore, in the last series of experiments using fat plus protein, the results are almost additive yet the fat was diluted to the same extent as when sugar was given. In all rats employed in this type of experiment, the oxygen consumption after protein alone was supplied proved to be lower than that obtained after feeding protein plus fat. This is just the opposite of the results found when sugar was given with fat or protein. Assuming protein catabolism to be equally large in both series of experiments, then the calorogenic effects of the mixture should amount to 21.2 per cent of the basal results if the individual effects are additive. It actually is 18.1 per cent. The difference may be due to a reduction in the S.D.A. of protein or fat or both. If due to protein, then its S.D.A. is less by 26 per cent. If due to fat, then the lowering of its S.D.A. is 36 per cent. Either of these figures is considerably smaller than the reduction found when sugar was mixed with fat (86 per cent) or with protein (about 59 per cent). It is apparent, therefore, that the S.D.A. of protein plus fat is much more nearly equal to the sum of the individual S.D.A.s than is either of the other combinations. The respiratory quotient also suggests no decided reduction in the S.D.A. of either component of the mixture. The calculated R.Q. for the mixture was 0.723 while the R.Q. found was 0.721. We should not attach too much importance to this evidence, however, since the R.Q.s obtained after ingestion of protein or fat are so close together that the R.Q. for the mixture could never be far from the calculated R.Q.

DISCUSSION. Joslin (1928) has pointed out that his diabetic patients between 1908 and 1914 had basal metabolisms which averaged 12 per cent above normal. This was before the treatment of such patients by undernutrition which of itself tends to modify metabolism. Numerous workers have also shown that depancreatized animals have an elevated basal metabolism (see Hedon, 1927, and Ring and Hampel, 1932). In 1935, Ring obtained evidence which suggested that this increased basal metabolism was brought about largely by the S.D.A. of fat. It now appears probable that the increased S.D.A. of fat observed was due to a reduction in the catabolism of carbohydrate. In the diabetic, the return of the basal metabolism to normal occurs only after sufficient insulin is given to stop the formation of ketone bodies and bring about the increased utilization of carbohydrate.

CONCLUSIONS

1. In rats, the specific dynamic action of 1.5 cc. of oleic acid plus 3 cc. of 50 per cent glucose ingested together (which elevated the basal metabolism 6.9 per cent during the eight hours after ingestion) is probably less than that of 1.5 cc. of oleic acid alone (8.7 per cent of basal figure).
2. The S.D.A. of 3 cc. of 50 per cent peptone plus 3 cc. of 50 per cent glucose (about 10.5 per cent of basal figure) is probably less than that of 3 cc. of 50 per cent peptone alone (about 12.0 per cent of basal figure) and is certainly less than the sum of the individual calorigenic effects (about 17.8 per cent of basal figure).
3. The S.D.A. of 3 cc. of 50 per cent peptone plus 1.5 cc. of oleic acid (18.1 per cent of basal figure) is almost equal to the sum of the S.D.A. of peptone ingested alone plus that of oleic acid (21.2 per cent of basal figure).

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THE INFLUENCE OF PROSTIGMINE, ATROPINE AND OTHER SUBSTANCES ON FIBRILLATION AND ATROPHY IN THE DENERVATED SKELETAL MUSCLE OF THE RAT

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From a previous study (1) of the biochemical characteristics of denervated skeletal muscle, at rest and after direct stimulation, it was concluded that the changes which were observed in paralyzed muscle could be accounted for by the continual fibrillatory twitching of the muscle fibers. In agreement with Langley (2) who based his conclusion on the observation of excessive oxygen consumption by paralyzed muscles as compared to normal resting muscles, it was suggested that the atrophy of denervation was an "overwork" atrophy.

This view is supported by our previous observation that the biochemical changes appear coincidentally with the onset of fibrillation, 4 to 5 days after denervation has been performed. It is at this same time that many workers including ourselves have noted that atrophy begins.

The previous evidence bearing upon the mechanism responsible for fibrillation and relating fibrillation to atrophy, has been recently reviewed in detail by Tower (3), and will not be repeated here. If the fibrillation of paralyzed muscle does indeed represent a chronic fatigue, with an insufficient rate of anabolism leading to atrophy, it would be expected that any means by which fibrillation could be increased would increase the rate of atrophy. Conversely, one might be able to minimize atrophy if one could decrease or abolish the fibrillation.

The present report deals with the results of our attempts to influence fibrillation and hence atrophy by various methods. The latter may be roughly divided into two categories, namely, specific and non-specific. The specific agents were selected on the basis of the previous work of Frank et al. (4) and Rosenblueth (5) which indicated that the fibers of denervated muscle contract in response to the minute amounts of acetylcholine normally present in the body fluids, to which normally innervated muscle fibers are completely insensitive. It seemed probable therefore that prostigmine, which would increase the activity of the acetylcholine present, would increase the fibrillation and hence the atrophy; while atropine which inhibits most of the actions of acetylcholine would tend to decrease atrophy. The non-specific agents were selected either because their known actions were related to the general excitability of muscle, or because they might be expected to favor synthetic metabolic processes in the muscle cells.

METHODS. The acute effects of various agents on the fibrillation of paralyzed

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muscle were noted by observing the exposed surface of the muscle under reflected light. In some experiments the action potentials were recorded by means of an amplifier system, although for our purpose this method offered no advantage over observation with the naked eye. A number of the materials which exhibited definite acute effects upon fibrillation were then tested as to their influence on muscular atrophy, in chronic experiments. Chronic experiments were also conducted with other materials of which the rationale is indicated below. For this purpose adult male rats weighing between 150 to 180 grams were used. They were maintained on our stock diet of "Purina" Fox Chow, supplemented with 10 per cent powdered skim-milk. Two animals were kept per cage during the experimental period. In each animal the sciatic and femoral nerves of one hind limb were cut. The contralateral unoperated limb served as the control. The degree of atrophy was judged by a comparison of the weights of the gastrocnemii at the end of 14 days.

Wet weights were used, since it is probable that the water content of denervated muscle does not vary significantly from the normal (3). Each animal was killed by a blow on the head, the intact whole gastrocnemii were carefully dissected and immediately weighed. The percentage atrophy in the untreated control animals was calculated as follows:

$$\text{Per cent atrophy} = \frac{(\text{weight of normal muscle}) - (\text{weight of denerv. muscle})}{(\text{weight of normal muscle})} \times 100$$

The effects of all procedures were evaluated by comparing the percentage atrophy in the gastrocnemii of the treated animals with the control figure.

RESULTS. The results of our acute experiments are briefly summarized in table 1. It will be noted that our predictions as regards the specific substances, prostigmine and atropine, were borne out. The former increased the fibrillation while the latter abolished it. A number of other substances also decreased the degree of fibrillation. Some of these substances such as the anesthetics were unsuitable for use in chronic experiments. Others such as quinine were found to be too toxic for chronic use, in the doses necessary to inhibit fibrillation. It was also realized that the influence of a particular substance on fibrillation might not necessarily be accompanied by a decrease in atrophy unless that substance were one which did not have any unfavorable effects on metabolic processes in general.

Table 2 summarizes the results of our chronic experiments as to the effect of various agents on the atrophy of denervated muscle. It may be seen that only the specific substances, prostigmine and atropine, caused significant variations from the control rate of atrophy. Prostigmine increased the atrophy by 47 per cent; atropine decreased the rate of atrophy by 39 per cent.

DISCUSSION. In view of the above results the evidence supporting a causal relationship between fibrillation and atrophy may be summarized as follows:

1. Paralyzed muscles begin to atrophy at the time of onset of fibrillation (6) (12).
2. The fibrillating paralyzed muscle consumes more O_2 than the resting normal muscle (13).

3. In frog muscle in which fibrillation does not occur after denervation, the rate of atrophy is exceedingly slow (14).

4. The onset of fibrillation in denervated rat muscle also marks the time of abrupt bio-chemical changes characteristic of fatigue or "overwork" (1). "Overwork" has been shown to be able to cause atrophy in normal muscles (15).

5. Fibrillation is probably due to an increased sensitivity of paralyzed muscle fibers to the normal minute amounts of acetylcholine present in body fluids. Prostigmine and atropine which affect the acetylcholine mechanism in opposite directions also affect the degree of atrophy correspondingly.

It seems necessary to attempt to reconcile the contradictory conclusions of Solandt and Magladery (6). These authors cast doubt on the causal relationship between fibrillation and atrophy for two chief reasons: 1. In their experi-

TABLE 1
Effect of various substances on the fibrillation of denervated rat muscle

	SUBSTANCE	DOSE	EFFECT	REMARKS
1	Prostigmine	5 mgm.	Noticeable increase	Also produces fibrillation and fasciculation of innervated muscles Effect obtained within several minutes
2	Atropine	10 mgm./ 100 grams	Inhibition	
3	Syntropan	10 mgm.	Questionable decrease	
4	Quinidine—SO ₄	10 mgm.	Inhibition	Effect obtained within several minutes
5	Quinine—HCl	10 mgm.	Inhibition	Effect obtained within several minutes
6	CaCl ₂	50 mgm.	None	Fibrillation diminished progressively and became significantly less only after about 30 minutes following induction of anesthesia
7	KCl	100 mgm.	None	
8	MgCl ₂	50 mgm.	None	
9	Avertin anesthesia		Questionable decrease	
10	Nembutal anesthesia		Inhibition	
11	Ether anesthesia		Inhibition	

ments barbitone tended to reduce the atrophy of denervated muscles without abolishing fibrillation, while quinidine, which did abolish fibrillation, had a sedative effect in the doses which they used. They therefore attributed the effect of quinidine to its sedative action, rather than to an action on fibrillation.

2. In many of their animals fibrillation was by no means a constant phenomenon in the paralyzed muscles, and there was no relationship between the degree of fibrillation during a given period and the amount of atrophy which occurred.

It is difficult to reconcile their observations as to the variability of fibrillation with most previous reports that fibrillation is a rather constant phenomenon until the substance of the muscle is completely wasted (3) (12). However, granting that their method of recognizing fibrillation was not at fault, their isolated records of fibrillation in a given muscle at various times can certainly not be

used as a quantitative index of the amount of work performed by the muscle fibers from the time of onset of fibrillation till the end of the observation period.

TABLE 2

Effect of various agents upon the degree of atrophy in the muscles of rats, 14 days after denervation

	SUBSTANCE USED	DAILY DOSE*	RATIONALE	NUMBER OF RATS	PER CENT ATROPHY	PER CENT DEVIATION FROM CONTROL
1	Stock diet			40	38	0
2	Prostigmine	5 mgm.	Inhibits the destruction of acetylcholine and thereby potentiates its action	15	56	+47
3	Atropine	15 mgm./100 grams	Decreases action of acetylcholine in most of its phases	21	23	-39
4	Syntropan	10 mgm.	Atropine-like drug	8	38	0
5	Quinine-HCl	5 mgm.	Inhibits fibrillation ((6) and table 1) (Proved very toxic for chronic use)	8	41	+8
6	Papaverin	10 mgm.	Speeds up recovery of cardiac muscle from fibrillation (7)	6	36	-5
7	CaCl ₂	100 mgm.	The Ca ⁺⁺ ion depresses the excitability of nerve and muscle	8	38	0
8	CaCl ₂ + Dihydro-tachysterol		To insure a continuous high Ca ⁺⁺ level in the body fluids	6	40	+5
9	Desoxycorticosterone + NaCl	2 mgm.	The K ⁺ ion plays a rôle in muscle excitability, and is related to acetylcholine action (10). Desoxycorticosterone depresses the K ⁺ level of the body fluids	6	41	+8
10	Desoxycorticosterone + KCl	2 mgm.		6	38	0
11	Thiamine	10 mgm.		6	39	+3
12	Pyridoxin (Vit. B ₆)	5 mgm.	Has a favorable action upon muscle fatigue in deficiency states (8)	8	37	-3
13	Tocopherol	3 mgm.	Cures certain experimental muscle dystrophies (9)	6	39	+3
14	Testosterone	1 mgm.	Depresses creatinuria, increases positive N balance and muscle mass (11)	6	35	-8
15	Progesterone	1 mgm.	Its actions resemble those of testosterone in some respects	6	36	-5
16	High carbohydrate diet		Favors glycogenesis, and provides part of substrate for protein synthesis	8	38	0
17	High carbohydrate diet + Protamine insulin	1 unit		8	35	-8

* The indicated dosage was begun on the day of denervation and administered daily until the animals were sacrificed.

Furthermore the graph in which they compared the effects of barbitone and quinidine on muscular atrophy does not bear out their conclusions. If the curve

for quinidine is extrapolated so as to make it comparable with that for barbitone at 14 days, it is apparent that the quinidine (which inhibited fibrillation) caused a significantly greater decrease in atrophy than the barbitone (which did not inhibit fibrillation) in spite of the fact that their hypnotic effects were similar.

From our results and the above considerations, it may be concluded that the fibrillation of denervated muscle leads to "overwork" atrophy. The degree of atrophy in paralyzed muscles has been increased and decreased by influencing the degree of fibrillation. It will be noted, however, that very large doses of atropine were needed to bring about the decreased atrophy in the paralyzed muscles of our rats. This would seem to deprecate its possibilities for therapeutic application over prolonged periods of time in humans, such as would be necessary in post-poliomyelitis paralyses. However the rat is notoriously resistant to the pharmacologic effects of atropine, and it seems not unlikely that the muscles of higher mammals and human beings might respond to much smaller doses. It seems worth while to test this possibility in monkeys.

CONCLUSIONS

1. On the hypothesis that fibrillation is the cause of atrophy in paralyzed muscles, various agents were tested for their influence on the degree of fibrillation in the denervated muscles of rats.

2. Of the substances which influenced fibrillation, and of a number of others used for different reasons, only prostigmine and atropine had any significant influence upon the rate of atrophy.

3. Prostigmine, which increased fibrillation, also increased the rate of atrophy by 47 per cent. Atropine, which diminished fibrillation, decreased the rate of atrophy by 39 per cent.

4. The possibility of the therapeutic application of atropine to prevent atrophy of paralyzed muscles in humans is raised.

We wish to thank Dr. Philip Lewin for his interest in and his encouragement of this work.

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THE NATURE OF QI AND QIII¹

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Q marks the beginning of the ventricular complex in many electrocardiograms. Its downward direction suggests that it is derived from early excitation in portions of the left ventricle, since electrical activity of this ventricle produces a downward deflection in the standard leads of the electrocardiogram (1, 2). The observation that lead I is formed by the summation of the anterior levocardiogram and the posterior dextrocardiogram, suggests that QI is derived from the anterior surface of the left ventricle. Since lead III records the summation of the anterior dextrocardiogram and the posterior levocardiogram, QIII should be derived from early activity in the posterior surface of the left ventricle (3). These inferences concerning the nature of QI and QIII were investigated in the following experiments. Twenty-one dogs were employed, and techniques were as previously described (1, 2).

THE NATURE OF QI. a. *Abolition of QI by application of potassium to the anterior surface of the left ventricle.* In experiments reported previously, it was noted that a Q wave was often found in the dextrocardiogram if it had been present in the control (1). In those experiments care was taken to avoid the septum in order to prevent the KCl solution from reaching the right ventricle. In the present experiments, the Q wave was reduced or abolished in lead I, when potassium was applied to cover the anterior surface of the left ventricle including the septum (fig. 1A).

b. *Change in amplitude of QI by thermal treatment of anterior left and posterior right ventricles.* According to the hypothesis presented in this paper, the downstroke of QI arises when a portion of the anterior left ventricle is discharged in advance of the posterior part of the right ventricle. The development of the posterior dextrocardiogram terminates the downward excursion of Q and produces the upward deflection which continues as the R complex. Variations in the interval separating the onset of the left and right ventricular components should alter the amplitude of Q just as it does the amplitude of R (2).

Heating the anterior left ventricle and cooling the posterior right ventricle should make the part of the left ventricle responsible for QI discharge earlier with respect to the right ventricle and increase the amplitude of QI. Conversely,

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cooling the anterior left and warming the posterior right ventricles should diminish QI by retarding the excitation of the left ventricle and hastening the

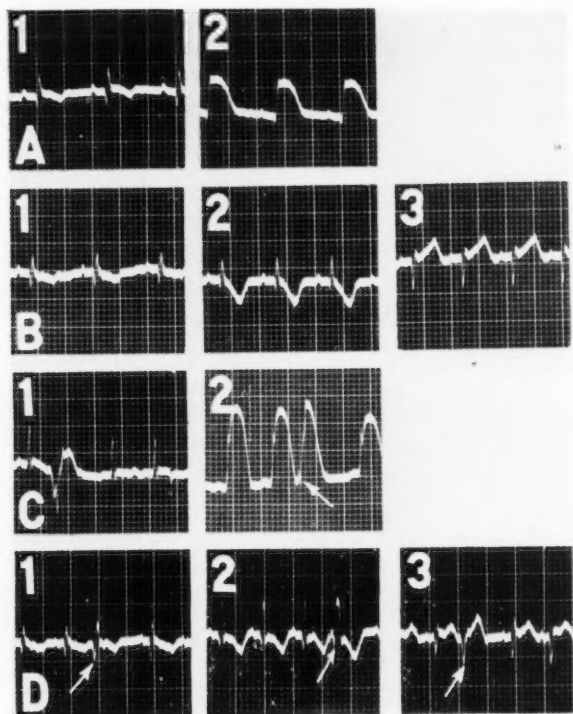


Fig. 1. Modification of QI. A. Dog, 17 kgm., 5/21/41. A₁ control, lead I. A₂, lead I after the anterior surface of the left ventricle was covered with a pledget soaked in M/5 KCl. Marked reduction of QI.

B. Dog, 8 kgm., 5/23/41. B₁. Control, lead I. B₂, warming right posterior and cooling left anterior surfaces of the heart. Reduction of amplitude of QI. B₃, cooling right posterior and warming left anterior surfaces of the heart. QI increased.

C. Dog, 10 kgm., 7/23/41. C₁. Lead I showing extrasystole elicited from a point just to the right of the anterior septum. Note large Q and small R. C₂, lead I after application of pledget soaked in M/5 KCl to the anterior surface of the left ventricle. QI practically abolished.

D. Dog, 5 kgm., 7/2/41. D₁. Control, lead I showing extrasystole elicited from a point 1 cm. to right of the anterior septum. The resulting complex shows Q and R closely resembling the normal complex. D₂, warming posterior right and cooling anterior left surfaces of the heart. QI reduced in extrasystole as well as in normal complex. D₃, cooling posterior right and warming the anterior left surfaces of the heart. QI increased in the extrasystole and in the normal complex.

discharge of the right ventricle. Fig. 1; B₁, B₂, B₃ illustrates the experimental confirmation of these inferences.

c. Production of QI in the ventricular extrasystole and its modification by polar-

sium and thermal changes. Extrasystoles elicited from the anterior septum show a downward initial deflection in lead I, with no upward component except T (4). Extrasystoles elicited from the center of the right ventricle show only an upward initial complex in lead I (2). As one moves the point of stimulation from the anterior septum toward the center of the right ventricle, the initial downward

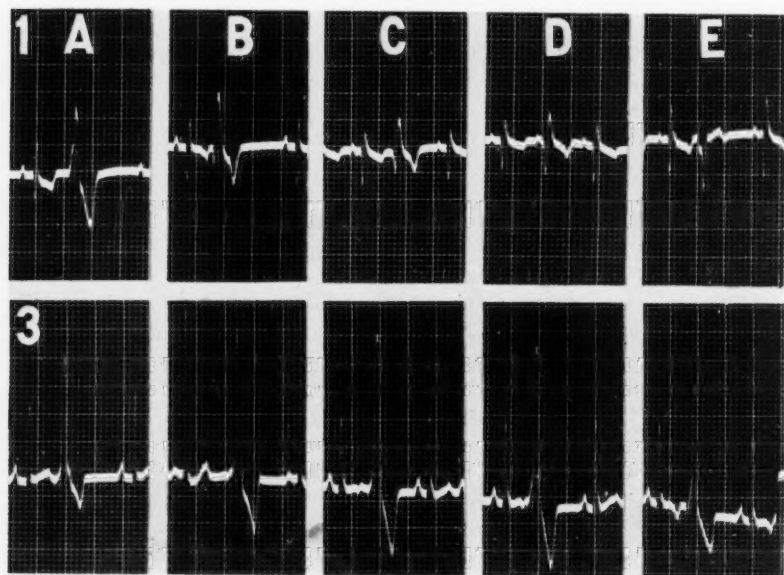


Fig. 2. Dog, 15 kgm., 9/24/41. Leads I and III. Extrasystoles in leads I and III elicited from points on the anterior surface of the right ventricle. A, extrasystoles in leads I and III elicited from the center of the right ventricle. The initial complex is upright in both leads. B, C, and D, extrasystoles elicited from points on the anterior surface of the right ventricle 1, 2 and 3 cm. from the point stimulated in A. These show the appearance of a Q wave which increases in amplitude as the anterior septum is approached. The increase in Q is accompanied by a reduction in the amplitude of R. E, extrasystoles elicited from a point overlying the anterior septum. RI has disappeared, and the entire initial complex is directed downward. No significant alterations in the configuration of QIII in this series. In E_I and E_I large stimulus artefacts are visible.

Attention is called to the necessity for fully inflating the lungs and approximating the edges of the wound in order to record these changes.

deflection becomes progressively smaller and finally disappears completely. Following the diminishing downward wave an upright deflection appears which increases progressively in amplitude. At these intermediate points, therefore, the initial complex possesses both Q and R waves (fig. 2).

The configuration of the anterior septal extrasystole suggests that in lead I the spread of the impulse involves the whole of the anterior left ventricle before it reaches the posterior right ventricle. In the case of the intermediate extra-

systoles it may be presumed that a portion of the left ventricle is still excited in advance of the posterior right ventricle, giving the initial downward deflection of Q. The posterior dextrocardiogram terminates the downstroke of Q and contributed its upstroke and that of the R complex. The greater the distance of the point of stimulation from the septum, the sooner will the posterior surface of the right ventricle be discharged, and the smaller will the QI become (fig. 2). Finally, when the center of the right ventricle is stimulated, so that the posterior surface of the right ventricle is excited before any part of the anterior surface of the left ventricle, Q is no longer present and the initial deflection is upright.

The explanation of QI in these extrasystoles should therefore be the same as for the normal QI. It is to be expected therefore that the Q wave in these extrasystoles should respond to potassium and to thermal effects exactly as does the normal Q. Figure 1, C and D show that *a*, QI of the extrasystole is in fact abolished by potassium treatment of the anterior left ventricle (C2); *b*, QI is increased by heating the anterior left ventricle and cooling the posterior right ventricle (D3), and *c*, QI is diminished or abolished by cooling the anterior left ventricle and warming the posterior right ventricle (fig. 1, D2).

THE NATURE OF QIII. It has been suggested above that QIII arises when a part of the posterior left ventricle becomes active in advance of any part of the anterior right ventricle. When this sequence of excitation occurs, the initial downward deflection of QIII should be derived from the downward deflection of the posterior levocardigram; the upstroke occurs when the anterior dextrocardiogram develops. This hypothesis was tested in the following experiments which are counterparts of those by which the nature of QI was studied, and which may be summarized as follows:

a. Abolition of QIII by application of potassium to the posterior surface of left ventricle. In experiments in which potassium was applied to cover completely the posterior surface of the left ventricle including the septum, QIII was abolished (fig. 3, A1, A2).

b. Change in amplitude of QIII by thermal treatment of posterior left and anterior right ventricles. QIII was increased by heating the posterior surface of the left ventricle and by cooling the anterior surface of the right ventricle (fig. 3, B1, B3). It was diminished by cooling the posterior left ventricle and by warming the anterior right ventricle (fig. 3, B1, B2).

c. Production of QIII in the ventricular extrasystole and its modification by potassium and by thermal changes. Extrasystoles elicited from the center of the right ventricle show only an upright initial deflection in lead III. As the point of stimulation is moved toward the posterior septum a small QIII appears which grows progressively in amplitude as the R portion diminishes. Finally, at the septum QIII alone remains and R has disappeared (fig. 4).

An extrasystole starting at the posterior septum must excite a large part of the posterior left ventricle before the anterior right ventricle is stimulated and therefore its initial complex in lead III is downward and is of maximum amplitude. As the point of stimulation is moved to the right, less and less of the

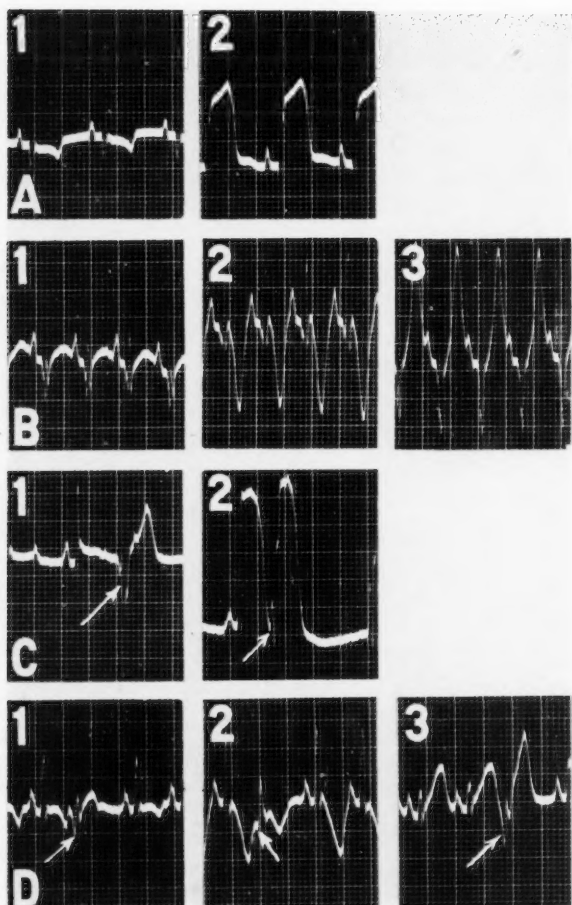


Fig. 3. Modification of QIII. A. Dog, 7.5 kgm., 5/10/40. A₁ control, lead III. A₂, lead III after application of pledget soaked in M/5 KCl to the posterior surface of the left ventricle; markedly reduced QIII.

B. Dog, 27 kgm., 7/28/41. B₁, lead III, control. B₂, reduction of QIII by warming anterior right and cooling the posterior left surfaces of the heart. B₃, QIII increased by cooling the anterior right and warming the posterior left surfaces of the heart. Thermal application has modified R and T as previously described.

C. Dog, 6 kgm., 7/18/41. C₁, lead III. Extrasystole elicited by stimulation of a point to the right of the posterior septum, showing a small Q and R and a large S. C₂, extrasystole after application of KCl to the posterior surface of the left ventricle. QIII absent, a small R and small S remain.

D. Dog, 11 kgm., 7/7/41. D₁, lead III showing extrasystole elicited from a point to right of the posterior septum. Definite Q and small R. D₂, elimination of Q in the extrasystole by warming the anterior right and cooling the posterior left surfaces of the heart. D₃, great increase in Q in the extrasystole caused by cooling the anterior right and warming the posterior left surfaces of the ventricles. The R and T waves of the extrasystole as well as of the normal complex show the alterations characteristic of heating and cooling.

posterior left ventricle is discharged in advance of any part of the anterior right ventricle, and results in progressive diminution in amplitude of QII.

In these experiments, QIII of the extrasystole was abolished by potassium applied to the posterior surface of the left ventricle (fig. 3, C1, C2); QIII was diminished by warming the anterior right ventricle and cooling the posterior left ventricle (fig. 3, D1, D2). QIII was increased in amplitude by cooling the anterior right ventricle and warming the posterior left ventricle (fig. 3; D1, D3).

DISCUSSION. The Q wave has been demonstrated to arise from the interference between initial portions of the dextro- and levocardiograms. When a

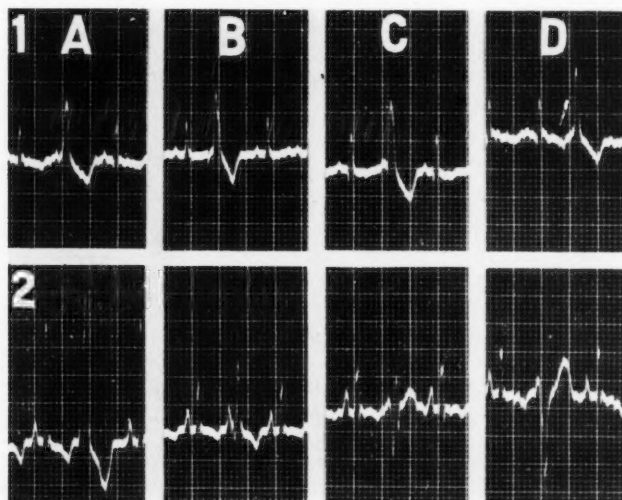


Fig. 4. Dog, 12 kgm., 9/26/41. Extrasystoles in leads I and III elicited from points on the posterior surface of the right ventricle. In lead I the complex is virtually unchanged in the series. In LIII the complex derived from stimulation of the center of the right ventricle (A) shows an upward initial deflection. As the point of stimulation approaches the posterior septum (B, C) a QIII appears which increases progressively in amplitude as the R wave diminishes. Finally at the posterior septum (D) the entire initial portion of the complex is directed downward.

portion of the anterior left ventricle is excited in advance of the posterior right ventricle, a Q appears in lead I, and its amplitude is dependent upon the interval which separates its component levo- and dextrocardiograms. The apex of Q marks the onset of the electrical events in the right ventricle, which arrest the further development of Q, and forms both the upstroke of Q and the upstroke of R. At the peak of R the remainder of the left ventricle becomes active and contributes the downstroke of R (2).

These studies require modification of the previous account (2) of the R complex only when a Q is present. In such a case activity in a small portion of the left ventricle precedes the sequence of excitation responsible for R. There is

evidence which indicates that the process of excitation may arrive at the surface of the left ventricle in the region of the septum before it reaches any other part of the surface of the heart (5, 6). This probably accounts for the observation that potassium must be applied close to the septum in order to diminish or abolish Q.

The fact that QI is modified by appropriate treatment of the anterior left and posterior right ventricles, supports earlier conclusions concerning the nature of lead I. Similarly, the modifications of QIII by treatment of anterior right and posterior left ventricles is in agreement with studies on the nature of lead III (3).

CONCLUSIONS

1. The downstroke of QI is produced by early electrical activity in the anterior surface of the left ventricle, and its upstroke results from excitation of the posterior surface of the right ventricle.

2. The downstroke of QIII is produced by early electrical activity in the posterior surface of the left ventricle, and its upstroke by excitation of the anterior surface of the right ventricle.

3. The presence of Q in the ventricular extrasystole has the same significance as Q in the normal complex. In lead I it results from primary activity in the anterior surface of the left ventricle and in lead III it arises from primary activity in the posterior surface of the left ventricle.

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THE PATHWAY OF SYMPATHETIC NERVES TO THE CILIARY MUSCLES IN THE EYE

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Because of the invariable accompaniment of pupillary dilatation when we have observed flattening of the lens in response to stimulation of the cervical sympathetic nerve in the cat, dog and rabbit (Olmsted and Morgan, 1939a; Morgan, Olmsted and Watrous, 1940; Olmsted and Morgan, 1941), it was a matter of interest to determine whether the sympathetic fibers to the ciliary muscle have the same origins and follow the same pathways as those to the iris.

The earliest observations showing the pupillo-dilator fibers have their origin in the spinal cord were recorded by Budge and Waller (1851) who found them to leave the cord in the 2nd thoracic nerves. It remained for Langley (1892) to map out precisely not only the origins of the sympathetic fibers to the iris, but also of those to the nictitating membrane and the eyelids. In the dog, cat and rabbit he found the pupillo-dilator fibers to be confined to the ventral roots of the 1st, 2nd and 3rd thoracic nerves, with minor differences in effectiveness of the three nerves in the different species; fibers for retraction of the nictitating membrane and opening of the eyelids had a slightly greater distribution, the first four thoracic and sometimes the 5th being effective. These sympathetic fibers to the eye after leaving the cord ascend in the cervical sympathetic to the superior cervical ganglion where a synapse is made. According to Duke-Elder (1938), the second neuron passes along the internal carotid artery in the carotid plexuses as far as the cavernous plexus. From here one of two pathways may be followed: 1, the fiber may join the nasal branch of the ophthalmic division of the trigeminal, leave it in the long ciliary nerves, and thus reach the eye via these nerves; or 2, it may pass to the ciliary ganglion as the sympathetic root of this ganglion, and leave it via the short ciliary nerves, presumably without the intervention of a synapse and the necessity of a third neuron, thus reaching the eye via the short ciliary nerves. Adler (1933, p. 37) states that these sympathetic fibers in the short ciliary nerves are pupillo-dilator, while Duke-Elder (1938, p. 309) believes that "they are essentially vasomotor."

Our method of tracing the pathway of the sympathetic fibers from the spinal cord to the ciliary muscle has been to disclose in cats, dogs, rabbits and rhesus monkeys, under nembutal or light ether anesthesia, the nerves known to carry pupillo-dilator fibers, or presumed to do so, and to submit these nerves to fairly

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weak unipolar faradic stimulation, observing any change in the curvature of the lens by skiascopic methods, and following pupillary changes by direct observation.

Spinal nerve roots. Since we experienced the same difficulties in keeping rabbits alive after exposure of the 1st thoracic nerves that Langley had found, we were unsuccessful in determining which of the spinal nerves carry the sympathetic fibers to the eye of this species.

In the cat, dog and monkey the cord was laid bare from the 7th cervical to the 4th thoracic nerves. The dura mater was not cut in order better to protect the exposed nervous tissue. The dorsal and ventral roots of each pair were disclosed and the dorsal roots cut between the cord and the ganglion in order to avoid reflex effects. In all three species we invariably found increase in hypermetropia upon stimulation of the ventral roots of the 1st thoracic nerves. In the cats and dogs under nembutal this amounted to 0.5 D-1 D. In the monkeys under nembutal and in cats under light ether anesthesia, the increase was over 1 D but less than 2 D. The results when the ventral roots of the 2nd thoracic nerves were stimulated were only slightly less consistent, the response being present in by far the greater number of trials in all three animals. Stimulation of the ventral roots of the 8th cervical in only one trial caused a change in the lens of the cat, and was ineffective in the dog; the 3rd thoracic was also ineffective in the cat and dog; but the 8th cervical and the 3rd thoracic were nearly always effective in the monkey (table 1).

That the effect on the lens was due to impulses passing peripherally over the ventral roots was shown by transecting the cord at the level of the 8th cervical nerves and again stimulating the ventral roots of the first two thoracic nerves. Flattening of the lens was still observable in all three species.

The distribution of sympathetic fibers to the ciliary muscle among the various spinal nerves is very similar to but may not be precisely identical with that of pupillo-dilator fibers. If we may use as a criterion the proportion of trials in which flattening of the lens was successfully elicited, we may infer that the range of these sympathetic nerve fibers in the spinal roots is only very slightly less than that of pupillo-dilator fibers, but the two sets of fibers would appear to be distinct from each other, since pupil dilatation was occasionally obtained without flattening of the lens (table 1), although as yet the converse has not been demonstrated. Comparison of the data furnished by Langley and by our experiments leads one to conclude that the sympathetic fibers to the nictitating membrane and the eyelids have a more extended origin in the spinal nerves, and those to the iris and to the ciliary muscle a more limited one.

Cervical sympathetic. The effect on the lens of stimulation of the cervical sympathetic in rabbits, dogs and cats we have reported elsewhere. It is to be borne in mind that we have found the lens to flatten upon stimulation of the cervical sympathetic whether or not the oculomotor nerve was cut intracranially, or whether or not the cervical sympathetic was severed from the cord. In the monkey under nembutal an increase in hypermetropia of 0.5 D to 1.25 D was observed upon stimulation of the cervical sympathetic. The effect is about the same in the monkey and cat, but rather less in the dog and rabbit. This agrees

with Cogan's (1937) results since he observed a change of 0.5 D to 0.75 D in the dog and about 1 D in the monkey.

To show whether, as in pupillo-dilator fibers, there is a synapse in the superior cervical ganglion for the fibers to the ciliary muscle, we painted the ganglion with 1 per cent nicotine. Stimulation of the 1st and 2nd thoracic ventral roots, which immediately before painting the ganglion had invariably produced the response, within 4 to 5 minutes after painting entirely failed to do so, and at the same time the pupillary response also failed. We may, therefore, conclude that the fibers to the ciliary muscle synapse in the superior cervical ganglion as do the pupillo-dilator fibers.

Long ciliary nerves. The two long ciliary nerves were disclosed in the cat and rabbit only. Stimulation of each of these two nerves caused an increase in

TABLE 1
Effect on pupil and lens of stimulation of ventral spinal nerve roots

	CAT		DOG		MONKEY	
	Pupil	Lens	Pupil	Lens	Pupil	Lens
C 7	0	0			0	0
C 8	Occasional response	Very occasional response	0	0	Almost invariable response	Almost invariable response
T 1	Invariable response	Invariable response	Invariable response	Invariable response	Invariable response	Invariable response
T 2	Invariable response	Almost invariable response	Invariable response	Invariable response	Almost invariable response	Almost invariable response
T 3	0	0	Occasional response	0	Almost invariable response	Almost invariable response
T 4	0	0	0	0	0	0

hypermetropia of slightly more than 0.5 D and also dilatation of the pupil. This was true whether the oculomotor nerve had been cut intracranially or the ciliary ganglion removed, or the short ciliary nerves destroyed.

Short ciliary nerves. Our experiments on the ciliary ganglion and the short ciliary nerves were also confined to the cat and rabbit. We followed the approach to this ganglion described by Shen and Cannon (1936). Stimulation of the ganglion caused a change in the lens in the direction of myopia and at the same time the pupil constricted. The same result was generally obtained, but to a much less degree, when individual short ciliary nerves were stimulated, 5 or 6 out of the 20-odd nerves having been isolated for stimulation in each animal. Stimulation of certain of the short ciliary nerves gave no response either of the lens or of the pupil. Presumably these particular nerves contained sympathetic fibers to blood vessels as suggested by Duke-Elder. There was at no time any indication of flattening of the lens or dilatation of the pupil. If, as Adler (1933, p. 37) states, "the short ciliary nerves contain both pupillo-constrictor and pupillo-dilator fibers," the constrictor effect entirely masked the dilator effect.

Similarly if there were sympathetic fibers to the ciliary muscle in the short ciliary nerves, their action was entirely masked by the overpowering action of parasympathetic fibers from the oculomotor.

In one cat, the ciliary ganglion was removed aseptically. One week later the eye on the operated side was more hypermetropic by 2 D than the normal eye on the other side. This condition still persists after 9 months. Under aseptic conditions the cervical sympathetic on this same side has been disclosed and stimulated. There was no increase in hypermetropia to be observed in the eye with the ciliary ganglion extirpated. This result should be compared with the well known finding that there is no pupillary response to light after cutting the oculomotor nerve. In this latter case, the pupil is so widely dilated that although the dilator fibers of the iris might theoretically relax in response to the light stimulus on the principle of reciprocal innervation, such relaxation (if it occurs) is unable to make an appreciable increase in pupil diameter (Gullberg, Olmsted and Wagman, 1938). The same appears to be the situation with regard to the ciliary muscle; the state of hypermetropia induced by extirpation of the ciliary ganglion is so great that no further increase is possible.

These results show that an almost identical pathway is followed from the spinal cord to the eye by sympathetic fibers for flattening of the lens and for dilatation of the pupil, the only differences being a very slightly less extensive origin of the former in the spinal nerve roots than of the latter, and, of course, termination in one case in the ciliary muscle, and in the other in the dilator fibers of the iris.

SUMMARY

Sympathetic fibers to the eye, stimulation of which causes the anterior face of the lens to flatten, emerge from the spinal cord in the ventral roots mainly of the first two thoracic nerves in the cat, dog, and monkey. In the monkey the 8th cervical and the 3rd thoracic ventral roots also contain these sympathetic fibers to the ciliary muscle. It has been demonstrated in the cat and rabbit that these fibers synapse in the superior cervical ganglion and that the post-ganglionic fibers reach the eye via the two long ciliary nerves. Whether the short ciliary nerves contain such fibers has not yet been demonstrated. These fibers to the ciliary muscle follow a pathway nearly identical with that for pupillo-dilator fibers.

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TRANSMISSION FATIGUE AND CONTRACTION FATIGUE

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Waller (1885) showed that a muscle which no longer contracted to nerve stimulation could respond when activated directly. Wedenski (1891) reported that upon indirect stimulation at high frequency the tension falls after an initial rise; a decrease of the stimulating rate then results in renewed contraction.

These observations suggested that neuromuscular fatigue was due to a junctional rather than to a muscular impairment. The seat of fatigue was much discussed at the beginning of the century (Woodworth, 1901, 1903; Joteyko, 1899, 1904).

Recent studies from this laboratory (see Rosenblueth, 1939, for references) have emphasized the existence of a junctional, transmission fatigue, resulting from relatively high frequency of indirect stimulation. In addition, Rosenblueth (*loc. cit.*) suggested that low frequencies of stimulation might lead to a fatigue of the contractile system—contraction fatigue—without involvement of the transmitting mechanism.

The fatigue of transmission (4th stage of transmission; Rosenblueth and Luco, 1939) is probably due to a gradual decrease of the quanta of acetylcholine released by the motor nerve impulses (Rosenblueth and Morison, 1937; Rosenblueth, Lissák and Lanari, 1939). Contraction fatigue could be due to the cumulative effect of the chemical residues of contraction and to the decrease of the energy-yielding materials in the muscle.

While the evidence for transmission fatigue is abundant, that for contraction fatigue is scanty. The present study was carried out with the purpose of learning whether the two modes of fatigue are independent, and securing further evidence regarding contraction fatigue.

METHODS. Cats were used, anesthetized with dial (Ciba, 0.75 cc. per kgm. intraperitoneally). A cannula was inserted into the trachea. The muscle employed was usually the gastrocnemius-plantaris, but some experiments were made on the soleus, tibialis anticus and peroneus longus muscles. When direct stimulation was employed the latter muscle was preferred because of its small volume. Drills were inserted into the tibia to fix the limb. The muscular contractions were recorded by attaching the tendon to the short arm of a writing lever pulling against rubber bands. The magnification was about 6-fold. Desiccation of the muscles was prevented by surrounding them with moist cotton pads.

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The sciatic nerves were cut on both sides, and either the popliteal or the peroneal branch was dissected in the upper part of the thigh for purposes of stimulation. The stimuli were condenser discharges with frequency regulated by a thyatron or a multivibrator circuit. The shocks were invariably maximal. The maximality was tested at various times in the course of the experiments by intensification of the stimuli; this intensification failed to increase the muscular response. The stimulating electrodes applied to the nerves were shielded silver wires. Direct stimulation was obtained through steel needles inserted into the tendon and the belly of the muscle.

RESULTS. A. *Fatigue at different frequencies of stimulation.* The description will deal mainly with the observations made on the gastrocnemius-plantaris

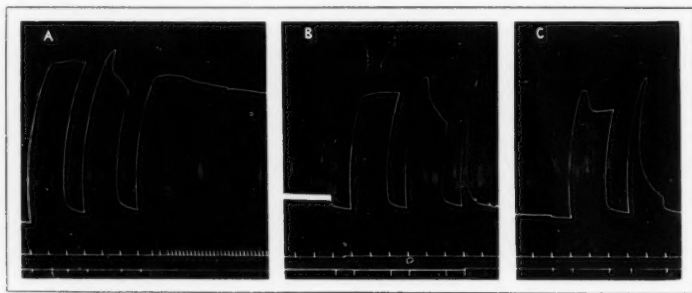


Fig. 1. Contraction fatigue without transmission fatigue. In this and the following figures the time signal marks 1-min. intervals. The lower signals mark the beginning and the end of the stimulation periods, or the transition from one to another frequency of stimulation. Unless otherwise stated the records are from gastrocnemius-plantaris and the stimulation is maximal and indirect (popliteal nerve, cut centrally).

A. Responses to 30 and 60 per sec. and beginning of response to 20 per sec. Between A and B the nerve was stimulated for 1 hour at 20, and then 1 hour at 10 per sec.

B. End of response to 10 per sec., responses to 30 and 60 per sec., and beginning of response to 120 per sec. Between B and C the nerve was stimulated for 11 min. at 120 per sec.

C. End of response to 120 per sec. and responses to 30 and 60 per sec.

muscle. The quantitative differences between the results from this muscle and those obtained from soleus and tibialis anticus are reported in section D.

Prolonged indirect stimulation at frequencies of 1 to 20 per sec. elicited single twitches or incomplete tetani. After the initial staircase the amplitude of the responses decreased. At first this decline was more rapid, later it was very gradual, so that a relatively constant amplitude could be maintained for periods of stimulation up to 8 hours.

In some observations short periods of rapid stimulation (30 to 120 per sec.) were interposed at different times during prolonged slow stimulation. Provided the high frequency periods were separated by relatively long intervals (over 10 min.), the responses to them were typical—i.e., the contractions, although smaller, were as well sustained as those which occur in rested muscles. Figure 1 illustrates a characteristic experiment.

With frequencies of stimulation around 30 per sec. the responses were as with the lower frequencies—i.e., well sustained for long periods (cf. fig. 2). A difference between these results and those obtained from rates of stimulation less than 20 per sec. was apparent, however, when brief tests at high frequency stimulation (60 to 120 per sec.) were interposed during a prolonged period of activation at 30 per sec. The responses to the test stimuli were then gradually less sustained than normally. A comparison of figures 1 and 2 illustrates the difference.

Similar but more striking results than those illustrated in figure 2 were seen when the higher frequency tests were made without a rest pause, by suddenly doubling the rate of the shocks from 30 to 60 per sec. in the course of a continuous response. The initial increase of tension corresponding to the change to a higher frequency became smaller and the subsequent relaxation of the muscle was faster and deeper with each successive test. Indeed, after long periods of

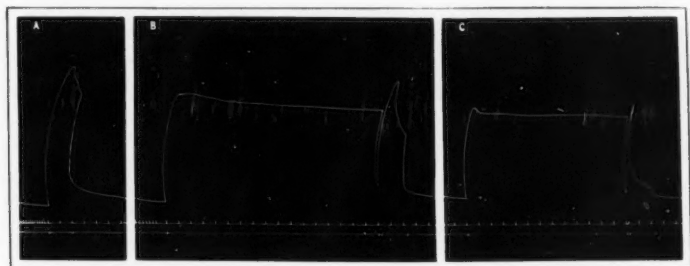


Fig. 2. Transmission fatigue produced by relatively low frequency of stimulation (30 per sec.).

A. Maximal stimulation of the popliteal nerve at the rate of 60 per sec.

B. Stimulation first at the rate of 30 per sec., then at 60 per sec.

C. As in B, but 20 minutes later. During this 20-min. interval several groups of stimuli were delivered as in B.

stimulation at 30 per sec., doubling the frequency resulted in only a minimal initial rise and mainly in a marked fall of tension.

Frequencies of stimulation between 40 and 150 per sec. caused unsustained tetanic responses. An initial high contraction was soon followed by a fall. The higher the frequency, the more prompt the fall and the lower the level attained after a given period of stimulation (fig. 3).

In some experiments brief periods of high frequency stimulation (60 to 120 per sec.) were repeated at regular intervals. When the rest intervals were short (e.g., 1 or 2 min.) signs of progressively increasing fatigue were evident (fig. 4A). This fatigue appeared first as a prompter than normal fall of tension in the course of stimulation and later as a decrease in the initial tension developed. If the intervals between the periods of stimulation were then lengthened a progressive recovery of the responses could be seen (fig. 4A).

Frequencies of stimulation higher than 150 per sec. were not used in this study. As shown by Rosenblueth and Cannon (1940) such higher frequencies cause a

sequence of falls and rises of tension (stages 2, 3a, 3b and 3c of neuromuscular transmission) that differ from the 4th stage (fatigue), with which this paper is concerned.

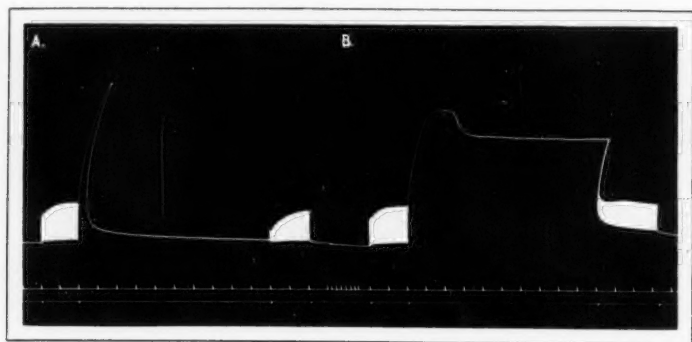


Fig. 3. Transmission fatigue as a function of the frequency of stimulation. Brief periods of low frequency stimulation (6 per sec.) precede and follow longer periods of stimulation at the following frequencies: A, 120 per sec.; B, 30 per sec.

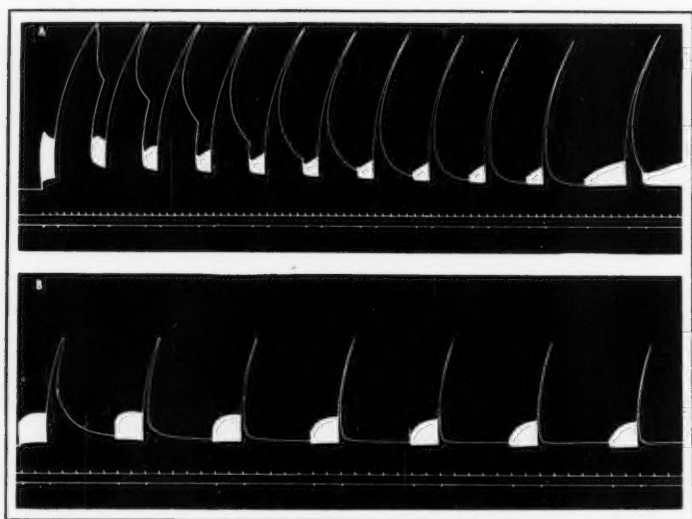


Fig. 4. Transmission fatigue obtained by repeated stimulation at a constant high frequency. Continuous stimulation of the popliteal nerve with alternating periods of low (6 per sec.) and of high frequency (A, 60 per sec.; B, 120 per sec.).

B. Recovery from fatigue. A fruitful method of studying fatigue is to observe the rate of recovery from a period of stimulation, in addition to observing the changes which take place during the action of the stimuli. Recovery was tested

by the application of slow (2 to 10 per sec.) shocks after a period of rapid frequency stimulation. The rate of recovery was influenced both by the frequency of the fatiguing rapid stimulation and by the length of the period during which such fatiguing stimuli were applied.

In figure 3 are illustrated tests of recovery made after stimulation for 10 min. with frequencies of 120 and 30 per sec. It is interesting to note that although the higher fatiguing frequency produced less tension than did the lower frequency the recovery of the test responses was quicker after slow than after fast fatiguing stimulation. When slow fatiguing frequencies were applied for a long time (several hours) the responses to slow test shocks were decreased and recovered only very gradually. There are thus two types of recovery, which suggest two types of fatigue. In one—high frequency stimulation—the decrease of the test twitches is marked, but recovery is prompt; in the other—low frequency fatigue—the decrease is relatively slight but the recovery is quite gradual.

In a series of observations a constant high frequency stimulation (e.g., 120 per sec.) was applied for different periods (e.g., 1 to 30 min.) and the recovery of responses to slow shocks was followed. The longer the period of high frequency stimulation, the slower was the recovery. Similarly, repeated applications of a high frequency stimulus, constant in rate and in duration, resulted in progressively slower recovery rates (fig. 4).

C. Independence of transmission and contraction fatigues. It is assumed in this heading that the fatiguing effects of high frequency stimulation (above 30 per sec.) are mainly junctional, whereas those of low frequencies (below 20 per sec.) are mainly muscular. This assumption will be justified later. On the basis of this interpretation the following observations reveal that the two fatigues are independent.

Progressively slower recovery of the responses to single shocks took place upon repeated applications of high frequency stimuli (increasing transmission fatigue), although the level of the twitches after recovery could be constant (fig. 4B; absence of contraction fatigue). Contraction fatigue may also be tested by direct stimulation of muscles. In figure 5 are shown the results of such direct stimuli during the course of a response to high frequency stimulation of the nerve. Transmission fatigue is indicated by the decline of the indirect response, while the absence of a concomitant contraction fatigue is revealed by the unvarying amplitude of the direct responses. In experiments in which contraction fatigue was caused by prolonged low frequency stimulation a later test analogous to that illustrated in figure 5 usually showed a recovery of contraction fatigue in the course of the development of transmission fatigue (see Waller, 1885).

These observations establish the possibility of producing transmission fatigue without contraction fatigue. The converse possibility is readily achieved. Prolonged stimulation at low frequencies causes, as shown before, a decrease of the amplitude of responses to test high frequency stimuli (contraction fatigue) with no change in the rate of fall of the test responses during stimulation (absence of transmission fatigue; cf. responses to 60 per sec. in fig. 1, A and B).

Recovery of transmission fatigue in the course of prolonged intense contraction is illustrated in figure 6. A series of periods of high frequency stimulation (120 per sec.) caused marked transmission fatigue. This fatigue was evidenced by the progressively slower rate of development of the responses to 30 per sec. (fig. 6A), a frequency that normally does not exhibit deficiency of transmission. Prolonged stimulation at 30 per sec. with only brief pauses (fig. 6B) then resulted in progressively better sustained responses.

D. *Differences between the soleus, gastrocnemius-plantaris and tibialis anticus muscles.* As is well known, these muscles differ quantitatively in the speed of their reactions so that soleus is a "slow" muscle, while tibialis anticus is "faster" than the other two.

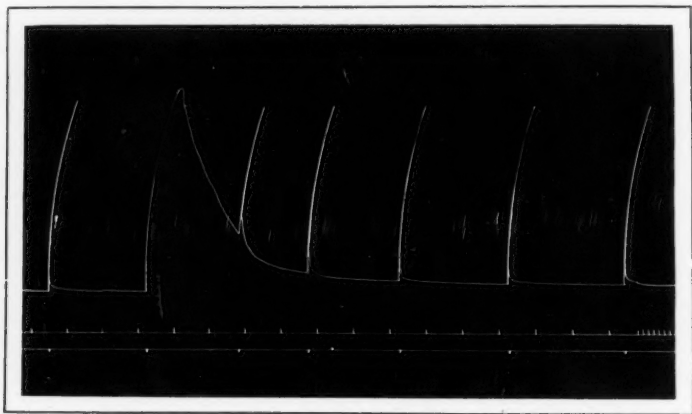


Fig. 5. Transmission fatigue without contraction fatigue. Peroneus longus muscle. The record shows the responses to direct maximal tetanic stimulation before (1st signal) and during (3rd to 7th signals) a period of indirect stimulation at 60 per sec. (beginning at the 2nd signal).

The main difference seen in these observations was that contraction fatigue was more prominent in the faster than in the slower muscles. Thus, experiments similar to that illustrated in figure 1 for gastrocnemius resulted in more striking decrease of tension when tibialis anticus was used. The responses to the high frequency tests did not reveal a decreased ability for sustained effects. The decrease of tension was therefore due to contraction, as opposed to transmission fatigue. Similarly, prolonged stimulation at low rates (e.g., 10 per sec.) caused only a slight fall of response in soleus, a moderate one in gastrocnemius, and a very prominent decline in tibialis anticus.

DISCUSSION. A monistic theory of neuromuscular fatigue fails to account for the data. The observations in sections A and B on the effects of prolonged stimulations at different rates show that there are marked differences between the effects of slow (20 per sec. or less) and of high (30 per sec. and more) frequencies. With the former the responses to all test frequencies are approxi-

mately equally depressed (fig. 1). After prolonged high frequency stimulation, on the other hand, the responses to slow frequency tests are only transiently affected, while the responses to high frequency tests are markedly depressed (fig. 4).

The facts thus suggest two different mechanisms for neuromuscular fatigue—one preponderant with low frequency stimulation, the other prevalent during high-rate indirect activation. The dualistic assumption stated in section C, that high frequencies depress transmission while low rates depress contraction, readily fits that suggestion.

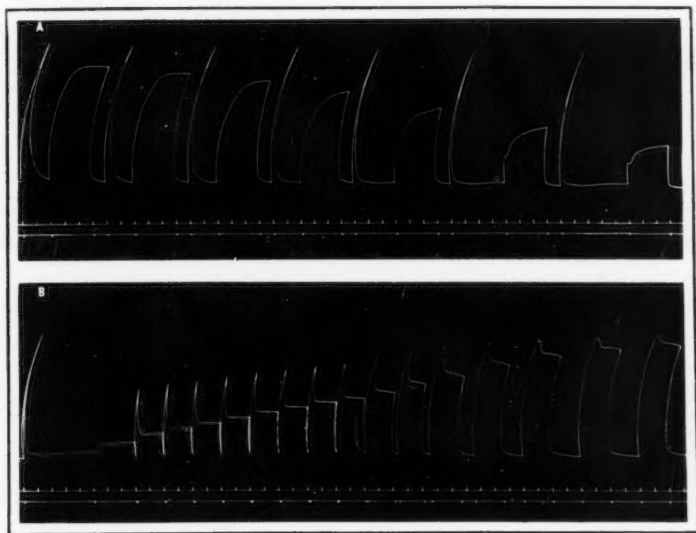


Fig. 6. Transmission fatigue produced by 120 per sec. followed by recovery at 30 per sec.

A. Series of responses to 120 per sec. followed without pause by 30 per sec.; a rest period of 1 min. separates the responses.

B. The record begins, 20 min. after A, with 120 per sec. The frequency was then changed to 30 per sec. as before. This frequency of 30 per sec. was then maintained except for the brief pauses shown by the signals.

Transmission fatigue would occur when, after the initial outburst from rapid stimulation, the rate of discharge of acetylcholine at the motor nerve endings continues to be greater than the rate of production (Rosenblueth and Morison, 1937). Assuming that the rate of production is relatively constant, the rate of liberation increases with the stimulating frequency. A critical frequency should exist, therefore, below which the nerves can replace, during the intervals between impulses, all the acetylcholine liberated by each impulse. Above that critical frequency of stimulation the liberation would exceed the production and a deficit would ensue, the greater the higher the frequency.

The theory accounts readily for the paradoxical effects of stimulation at 30

per sec. in different experimental conditions. Normally the responses to this frequency do not show any prompt drop of tension, indicative of a decrease in the number of active fibers, and therefore indicative of transmission fatigue. That 30 per sec. will cause transmission fatigue, however, is shown by the responses to tests with higher frequencies (fig. 2). To account for "subliminal" transmission fatigue it is sufficient to assume that the amounts of acetylcholine released normally are well over the threshold quantities. Stimulation at 30 per sec. would then cause some decrease of the acetylcholine output, but the quanta would still be above the threshold of the discharging muscle fibers. Recovery of the transmission fatigue produced by previous high frequency stimulation during the time of application of 30 per sec. is likewise explained by the theory. High frequency stimulation lowers the acetylcholine contents of the nerves to subthreshold levels. The equilibrium between production and liberation at 30 per sec. is suprathreshold. If 30 per sec. is applied after a higher frequency, therefore, the acetylcholine concentration will slowly rise from a subthreshold to a suprathreshold level (fig. 6).

The theory outlined for transmission fatigue fails to account for the fall of tension after the initial high rise in figure 6B upon application of 30 per sec. after a brief pause. Many factors probably at play have been deliberately dismissed in the previous discussion, for purposes of simplification. Thus, the muscles have been considered uniform, whereas it is known that they contain "slow" and "fast" fibers with quantitatively different properties. Post-tetanic effects of previous stimuli (see Rosenblueth and Morison, 1937, for references) have been neglected. How much these factors may modify the results is difficult to evaluate. Without invoking them it is possible to account for the phenomenon in question by one of several alternative subassumptions—e.g., by postulating that the rate of production of acetylcholine is not constant, but is a function of the level of mediator present at a given time—but such subassumptions would be entirely speculative.

If contraction fatigue is due to metabolic chemical changes, as is likely, its degree should be correlated with the amount of contraction and its recovery should be slow. These inferences are supported by the observations. Some degree of contraction fatigue should take place even when stimulation is carried out at high frequencies, since the muscles contract, except in such experiments as those of Luco and Rosenblueth (1939) where curare abolished contractions. Circulated mammalian muscles are, however, surprisingly resistant to contraction fatigue (see Asher, 1923). It was possible to obtain responses sustained at a relatively high tension for several hours by use of adequate frequencies of stimulation (p. 765).

The independence of the two modes of fatigue studied is clearly shown in the observations mentioned in section C (figs. 1, 5 and 6). This independence is not astonishing since the two processes which lead to a depression of the responses take place at different points in the neuromuscular system. Indeed, if the theory of Rosenblueth and Morison (1937) for transmission fatigue is accepted this fatigue is nervous in location, whereas contraction fatigue is properly muscular.

SUMMARY

The results of indirect maximal stimulation at different frequencies of several circulated muscles of the cat were studied (figs. 1 to 6).

The fatigue caused by frequencies of stimulation higher than about 30 per sec. (figs. 4 and 6) is interpreted as due to a deficiency of transmission (p. 767); that resulting from stimulation at low frequencies (below 20 per sec.; fig. 1) is interpreted as due to a deficiency of the contractile system (p. 767). At frequencies between 20 and 30 per sec. "subliminal" transmission fatigue is present (fig. 2; p. 770).

Recovery of transmission fatigue is relatively prompt (figs. 3 and 4), that of contraction fatigue is slow (p. 766).

Transmission fatigue and contraction fatigue are independent phenomena (figs. 1, 5 and 6; p. 767).

Contraction fatigue is more prominent in "fast" than in "slow" muscles (p. 768).

Transmission fatigue is discussed from the standpoint of Rosenblueth and Morison's (1937) theory of decrease of acetylcholine output (p. 769). Contraction fatigue is attributed to metabolic changes at the muscles (p. 770).

I am very indebted to Dr. W. B. Cannon and Dr. A. Rosenblueth for their suggestions and valuable criticism.

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BLOOD VISCOSITY UNDER DIFFERENT EXPERIMENTAL CONDITIONS AND ITS EFFECT ON BLOOD FLOW¹

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According to Poiseuille's law, fluid viscosity limits the flow of liquids through small tubes by an amount which varies with several factors. Because of the nature of the vascular bed it is therefore of interest to determine in experimental animals the differences in the viscosities of blood normally existent, or those induced by various experimental procedures involved in the measurement of blood flows, and to quantitate the degree to which such differences may influence the facility with which blood may pass through the vascular beds of experimental animals. Since the extent of such information is limited, an experimental study of viscosity effects has been made.

In the application of Poiseuille's law, the specific viscosity of water and many fluids is independent of the characteristics of the apparatus used. However, the properties of blood are such that the apparent viscosity varies widely with the pressure applied, the volume of fluid measured, and the bore of the capillary tube; hence, the values are empirical (1, 2, 3, 4). For our purpose, values obtained with any one set of conditions for different bloods are acceptable, since the chief interest lies in the relative viscosity of the bloods, which can be determined with reasonable accuracy by a number of methods.

For determining blood viscosity the apparatus consisted of a glass capillary tube of 0.5 mm. bore by 14 cm. long fused to a tube of 2.5 mm. bore by 13 cm. long, suspended in a water bath at about 20 degrees with the horizontal. A constant suction of 36 mm. Hg was applied to the larger end, and the ratio of the times required for blood and water, respectively, to traverse the tube to a fixed and predetermined mark was regarded as the specific viscosity of the blood. In general, for the control determinations 1 to 2 cc. of venous blood was withdrawn from a dog by a syringe (without anti-coagulant) and placed immediately in a small cup under the suction tube for determination of viscosity. The total time for blood withdrawal and viscosity determination was generally less than one minute. In routine use duplicate determinations agreed within 5 per cent. In some experiments the hematocrit was also determined.

Typical values for hematocrit reading and blood viscosity in dogs under a variety of experimental conditions are shown in table 1, part A. The blood

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viscosity values found in normal, unanesthetized dogs varied from 3.7 to 7.1 with an average of 5.1. While morphine sulphate and defibrination have no signifi-

TABLE 1

Blood viscosity studies

A. Typical specific blood viscosities under different experimental conditions

NUMBER OF EXPERIMENTS	CONTROL VISCOSITY	CONTROL HEMATOCRIT	VISCOSITY AFTER VARIABLE	HEMATOCRIT AFTER VARIABLE	PER CENT CHANGE IN VISCOSITY	CONDITIONS
15	5.07	40.3%				Normal unanesthetized dogs
15	6.9	50.6				Normal unanesthetized dogs
6	5.9	—	5.8	—	-0.1	60 minutes after 30 mgm. morphine sulfate subcutaneously
15	6.9	50.6	5.6	47.5%	-18	60 minutes after 30 mgm. sod. pentobarbital/kilo
5	5.07	40.3	5.53	46.0	+9.1	Ether anesthesia
4	4.2	—	4.3	—	+0.2	Heparin in vivo 100-200 units/kilo
4	7.4	—	7.2	—	-0.2	Heparin in vitro 20 units/cc.
6	3.67	30.67	3.77	30.6	+0.2	Defibrinated blood
4	5.20	44.6	4.47	39.2	-14	3 hrs. after 300 cc. hemorrhage
6	5.20	44.6	6.43	36.6	+23	Sodium citrate 1% <i>in vitro</i>
7	4.3	33.1	5.2	31	+20	Pontamine fast pink in vitro 2 mgm. per cc.
7	4.3	33.1	5.5	30	+23	3 mgm. per cc.
7	3.67	30.6	4.67	36.0	+27	Pontamine fast pink in vivo 200 mgm./kilo
5	4.4	—	5.0	—	+13	Temperature changed from 38°C.-33°C.

B. Effect of changes in blood viscosity on blood flows

NUMBER OF EXPERIMENTS	MM. Hg		SPECIFIC VISCOSITY			BLOOD FLOW—CC./MIN.			CONDITIONS
	Perfusion pressure	Mean B. P.	Control	Variable	Per cent change	Control	Variable	Per cent change	
11	129	115	5.7	3.0	47	19	46	142	Femoral bed perfused in anesthetized dog. Anticoagulant—heparin and pontamine fast pink. Viscosity changed by adding cells or plasma
	129	90	5.7	3.0	47	31	58	87	Same as above
	74	—	6.6	3.3	50	28	46	55	Same as above
	110	90	5.7	3.7	35	47	64	36	Carotid bed perfused in anesthetized dog. Anticoagulant same. Viscosity changed by adding pontamine fast pink

cant effect on blood viscosity, sodium pentobarbital reduces the viscosity considerably and ether anesthesia alone causes a marked increase which varies directly with the depth of anesthesia. Of the anti-coagulants, heparin added

either to drawn blood or injected by vein into unanesthetized or anesthetized dogs, has never been observed to alter significantly the blood viscosity, while sodium citrate added to drawn blood increases viscosity greatly. However, the most striking effects are produced with pontamine fast pink (and chlorazol fast pink) which cause very large increases in viscosity. In dogs anesthetized with sodium pentobarbital, this effect may be partially neutralized. In the range of body temperature an average of one degree centigrade drop in temperature increases viscosity by about 2 per cent.

In most instances *in vivo* variations in specific viscosity are directly related to the hematocrit reading and red cell count (cf. table 1 for typical values). Of interest, however, is the fact that blood viscosity and the hematocrit vary inversely when sodium citrate, pontamine fast pink (or chlorazol fast pink) are added to drawn blood. The mechanism for this increase in viscosity apparently lies chiefly in the red cells since the viscosity of the plasma does not change significantly. For example, when 5 mgm. of pontamine fast pink per cubic centimeter was added to whole blood, the viscosity increased from 5.2 to 7.1, the hematocrit decreased from 44.6 to 37.9, while the viscosity of the plasma changed only slightly, 2.0 to 2.3. If fresh plasma is added to the mixture of dye and cells, the viscosity still remains high (6.68) and the hematocrit increases to 41.7. Finally, when the cells are washed to remove the dye and are then suspended in fresh plasma (without dye) both the hematocrit reading and the viscosity value approach the original. Likewise, the addition of pontamine fast pink to a mixture of cells and saline increases the viscosity from 3.5 to 5.3. Just how the same anti-coagulant such as pontamine fast pink, *in vivo* increases the hematocrit, red cell count and viscosity, while *in vitro* increases the viscosity but decreases the hematocrit with the same cell count, is at present not explained.

These data indicate that the blood viscosity of different dogs is fairly high, varies widely, and can be grossly altered by experimental procedures used in the measurements of blood flow. The findings regarding the blood viscosity existing after defibrination differ from those of Burton-Opitz, while the effects of morphine, ether, hemorrhage, and temperature change agree in general with those found by this author (5). Possibly the difference may be related to the number of red cells removed in the process of defibrination.

Using the constant pressure meter, Gregg and Green (6) found that gross changes in viscosity obtained by substitution of Locke's solution for a dog's own blood increased left coronary inflow by 300 to 400 per cent. Presumably, therefore, changes in viscosity of the magnitude observed here should alter by a significant amount the rate of blood flow through various vascular beds. To test this, the rates of flow of homologous bloods of different viscosities into typical vascular beds in anesthetized dogs were compared. The blood was heparinized, divided, and its viscosity adjusted by adding or subtracting cells, or in some instances by the addition of pontamine fast pink, so that its viscosity equaled, exceeded, or was less than that of the recipient dog. The bloods were placed in bottles under a constant pressure head a few millimeters Hg greater than the mean blood pressure and connected to the peripheral end of a rotameter (7) which in turn was connected to the peripheral end of a carotid or femoral artery.

In some instances the kidneys of dogs were removed and perfused at a constant pressure through the renal artery by means of a pump system. In some of these experiments (especially the femoral perfusions) to secure more constant readings, most of the collateral flow was blocked during the flow reading. In both set-ups, by appropriate stopcocks, the vascular bed could be alternately perfused with bloods of different viscosities and the rate of flow determined immediately from the calibrated rotameter. An average decrease in viscosity of one per cent was found to increase the flow by 1.4 per cent (table 1, part B, for typical experiments).

These results indicate that differences in viscosity, normally existent in different dogs or induced by different experimental procedures, may exert a large influence on blood flows. Hence, the interpretation or comparison of blood flow values obtained in different dogs with or without aid of anesthesia and anticoagulants should be tempered by recognition of the influence of viscosity on such values.

SUMMARY AND CONCLUSIONS

By means of a simple viscometer (in which duplicate determinations agree within 5 per cent) the specific viscosity of blood has been determined empirically in normal unanesthetized dogs and in dogs after the induction of experimental procedures preliminary to the measurement of blood flows.

In normal dogs, the specific viscosities have been found to vary from 7.1 to 3.7. Blood defibrination, the addition of heparin (*in vitro* or *in vivo*) and the injection of morphine sulphate do not affect the viscosity. Hemorrhage (after a short period) and barbital anesthesia cause considerable reduction in viscosity; a decrease in blood temperature increases viscosity, while ether anesthesia, the *in vitro* use of sodium citrate, and the *in vitro* or *in vivo* use of pontamine fast pink (or chlorazol fast pink) give large increases in viscosity. In general these changes in viscosity are directly related to the hematocrit. However, in the case of pontamine fast pink and sodium citrate added to drawn blood the relation is reversed.

Experiments show that these changes are sufficient to alter greatly the facility with which blood flows through vascular beds of the anesthetized dog or through perfused organs.

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EFFECT OF BILE AND BILE SALTS ON ABSORPTION OF SODIUM OLEATE FROM JEJUNAL LOOPS OF DOGS¹

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The statement is usually accepted that bile acids enhance the absorption of fat and fatty acids (1). These experiments were undertaken to find which of the common bile acids would have a greater effect on absorption. Soon after the experiments were begun, Riegel, Elsom and Ravdin (2) reported that bladder bile, liver bile and sodium taurocholate were all effective in promoting absorption of oleic acid. They found, too, that sodium taurocholate was somewhat more effective in promoting absorption of oleic acid than was liver bile or gall-bladder bile. They used bile in quantities that furnished the same amount of taurocholate that they used in the taurocholate experiments. Our experiments were designed to show what the effect of bile, sodium taurocholate, sodium glycocholate, and sodium desoxycholate would be on the absorption of oleic acid.

EXPERIMENTAL. Our preliminary experiments were done using a Thiry fistula, and next a Johnston (3) loop. Neither of these proved entirely satisfactory, so a new type of closure for intestinal loops was devised (4). This new loop made quantitative introduction and removal of samples quite easy, and the closure was absolutely tight.

One cubic centimeter of oleic acid and 29 cc. of water were introduced into the loops in preliminary tests, but this material was irritating, so sodium oleate was used as our test material, and our measure of absorption in all the experiments reported herein was made on sodium oleate. Tap water was used at first to irrigate the loops. Later saline was used, since Dennis (5) reported the possible toxic effect of distilled water on intestinal mucosa. No difference has been apparent in the two procedures. The loops were washed out with 4 separate 20 cc. volumes of saline before experimental periods.

To measure the degree of absorption of sodium oleate when it was introduced alone, sodium oleate solutions were introduced into the loop and enough water was added to make the total volume 30 cc. The material was permitted to remain in the intestine 3 hours. The loops were then drained and rinsed 4 times with 20 cc. volumes of saline. The saline washings were combined with the material drained from the loop for analysis. So the data might be easily com-

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parable with those of Riegel et al. (2), an attempt was made to use 10 cc. of 10 per cent sodium oleate in 30 cc. of solution. It was observed that such quantities caused a large flow of fluid into the loops, so the attempts to obtain closely comparable data were abandoned. The quantities of sodium oleate used in experiments in this paper were 500, 200 and 400 mgm., respectively. This meant that the concentrations of oleate in the 30 cc. of fluid introduced were 1.67, 0.67 and 1.33 per cent.

To test the effect of bile and bile salts on absorption of oleate, sodium oleate solutions were introduced into the loops with bile or whatever bile salt solution was to be tested, and the total volume made up to 30 cc. with water. Technical sodium taurocholate (Eastman) was used in most of the experiment. A few earlier experiments with synthetic sodium taurocholate (6) gave results quite comparable with those obtained with the technical material. The quantity used contained 257 mgm. of taurocholate, based on the sulfur content of the material. The quantity of bile used was that which contained 257 mgm. of taurocholate (from 2 to 4 cc. of gall-bladder bile from dogs). The glycocholate was equimolar, with respect to bile salt content, with the taurocholate used, so all 3 solutions were equimolar with respect to bile salts. Sixteen experiments were carried out on 7 dogs. When several experiments were done on the same animal, at least 2 weeks elapsed between experiments.

Analyses were made as follows: the fluid obtained from an absorption experiment was acidified with hydrochloric acid in a separatory funnel. This was extracted with 3 separate 80 cc. portions of petroleum ether. The 3 fractions were combined and washed with about 150 cc. of water. The ether layer was then centrifuged, and any water siphoned from the bottom. Anhydrous sodium sulfate was added, the material shaken and again centrifuged. This procedure removed the water and the solid material which tended to collect at the interface between the ether and water layers. After filtration the ether was allowed to evaporate, the oily residue taken up in 25 cc. of alcohol, and titrated with tenth-normal sodium hydroxide. The difference between this titration and that obtained by a similar procedure on a quantity of oleate equal to that introduced into the loop was considered to be a measure of absorption of oleate by the dog.

RESULTS. The data shown in table 1, obtained after using 500 mgm. of sodium oleate, indicate that it was absorbed to a considerable extent when left in the intestine for 3 hours, and was absorbed to a greater extent when bile was introduced with the oleate. Introduction of sodium taurocholate with the oleate decreased the per cent of absorption, rather than increasing it. Sodium glycocholate, not a normal constituent of dog bile, also failed to increase the per cent of absorption above that which occurred when oleate alone was introduced into the loop. In order to find whether a lower ratio of fatty acid to bile acid would enhance the absorption of fatty acid, a second group of experiments was carried out using only 200 mgm. of sodium oleate, but keeping the quantities of bile salts constant.

The experiments with 200 mgm. of oleate were less satisfactory than those in which 500 mgm. or 400 mgm. of oleate were used, for the absolute values

TABLE 1
Per cent of sodium oleate absorbed from jejunal loops of dogs after introduction alone
or in combination with bile or bile salts*

Per cent of oleate absorbed

MATERIAL INTRODUCED	DOG 1	DOG 2 EXPT. 1	DOG 2 EXPT. 2	AVERAGE
500 mgm. sodium oleate	28.3 ± 2.2§ (2)†	31.3 ± 4.2 (7)	35.4 ± 4.6 (6)	30.8
500 mgm. sodium oleate + 257 mgm. sodium taurocholate	25.5 ± 6.6 (3)	15.2 ± 2.7 (4)	23.0 ± 16.7 (3)	22.9
500 mgm. sodium oleate + 236 mgm. sodium glycocholate	22.4 ± 4.2 (2)	27.2 ± 5.2 (6)	34.2 ± 1.4 (2)	26.6
500 mgm. sodium oleate + bladder bile containing 257 mgm. sodium taurocholate	63.1 ± 6.5 (3)	38.6 ± 8.8 (3)	47.3 ± 2.2 (3)	53.0

MATERIAL INTRODUCED	DOG 3	DOG 4	DOG 5	DOG 6 EXPT. 1	DOG 6 EXPT. 2	DOG 7 EXPT. 1	DOG 7 EXPT. 2	AVERAGE
200 mgm. sodium oleate	8.2 ± 3.5 (3)	60.5 ± 12.8 (3)	52.3 ± 4.9 (5)	49.1 ± 2.3 (3)	72.8 ± 1.1 (6)	43.9 ± 4.4 (3)	77.7 ± 3.7 (6)	48.5
200 mgm. sodium oleate + 257 mgm. sodium taurocholate	0.0 ± 0.0 (3)	33.6 ± 9.8 (3)	52.2 ± 7.9 (4)	0.0 ± 0.0 (3)	31.6 ± 5.9 (6)	22.8 ± 6.8 (3)	36.8 ± 8.1 (3)	26.3
200 mgm. sodium oleate + 236 mgm. sodium glycocholate	23.4 ± 2.9 (3)	0.0 ± 0.0 (1)	51.7 ± 16.7 (3)	55.3 ± 5.4 (3)	64.6 ± 4.6 (3)	33.8 ± 10.6 (3)	39.5 ± 14.7 (3)	34.4
200 mgm. sodium oleate + bladder bile containing 257 mgm. sodium taurocholate	39.8 ± 14.6 (3)	48.7 ± 12.9 (3)	59.0 ± 6.6 (4)	58.9 ± 2.2 (3)	68.3 ± 3.0 (3)	43.0 ± 13.8 (3)	78.3 ± 4.7 (3)	54.2

MATERIAL INTRODUCED	DOG 6 EXPT. 3	DOG 6 EXPT. 4	DOG 6 EXPT. 5	DOG 7 EXPT. 3	DOG 7 EXPT. 4	DOG 7 EXPT. 5	AVERAGE
400 mgm. sodium oleate	46.7 ± 3.1 (3)	40.3 ± 4.1 (3)	57.4 ± 4.1 (6)	41.2 ± 1.6 (3)	56.3 ± 2.6 (3)	49.8 ± 1.8 (6)	48.6
400 mgm. sodium oleate + 257 mgm. sodium taurocholate *	5.5 ± 1.9 (3)	17.3 ± 3.1 (3)	30.3 ± 1.7 (3)	23.7 ± 2.0 (3)	54.5 ± 2.2 (3)	36.2 ± 8.0 (3)	27.9
400 mgm. sodium oleate + 236 mgm. sodium glycocholate	28.9 ± 4.4 (3)	41.1 ± 2.4 (3)	44.1 ± 4.6 (3)	48.9 ± 5.6 (3)	42.8 ± 0.0 (3)	44.9 ± 6.9 (3)	41.8
400 mgm. sodium oleate + bladder bile containing 257 mgm. sodium taurocholate	56.7 ± 1.7 (3)	72.7 ± 4.6 (3)	74.4 ± 3.8 (3)	67.9 ± 8.0 (3)	72.3 ± 7.8 (3)	77.4 ± 6.9 (3)	70.2
400 mgm. sodium oleate + 128.5 mgm. sodium taurocholate + 118 mgm. sodium glycocholate		26.0 ± 2.7 (3)	32.0 ± 2.4 (3)	44.1 ± 5.0 (3)	40.3 ± 2.7 (3)		35.6

* The oleate used was an aqueous solution. The bile salts were contained in 2 cc. of solution. Enough water was added to make the total volume introduced up to 30 cc.

† Averages obtained by using only 1 value for each dog.

‡ Number of experiments entering into mean.

$$§ \text{ S.E. of mean } = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

were small. For example, the quantity of tenth-normal sodium hydroxide required to titrate the acid removed from the loop of dog 7, experiment 2, averaged less than 1 cc. The larger percentage variations in experimental data for this group should probably have been expected on this basis. The general results of this group, however, were the same as those found after using 500 mgm. of oleate, namely, absorption of oleate alone, a greater absorption when bile was admixed, and an inhibition of absorption by bile salts.

A third group of experiments was then carried out using 400 mgm. of oleate. The results were quite in accord with the general findings of the first 2 groups. In addition, mixtures of taurocholate and glycocholate were used, equimolar with the bile salt solutions already mentioned. These mixtures gave no evidence of enhancing absorption of oleate.

The number of experiments performed with the several dogs was not always the same, so the 3 sets of average values given for different quantities of oleate used were obtained by using only 1 value for each animal on that procedure. This avoided the error of permitting values from one animal to overbalance the others. Since not all the dogs responded similarly in a given series, averaging values may be open to criticism.

Each dog excreted a considerable quantity of fluid from the loop daily when it was not undergoing absorption experiments. Although not measured, these volumes were observed to be considerably larger during the weeks of experimentation than during non-experimental periods. The decline in volume excreted from the experimental to the non-experimental periods required several days. Such activity may have been due to the activity of a functioning loop, as compared to a non-functioning loop, during the quiescent periods.

DISCUSSION. Peters (7) has reported that taurocholate, in concentrations somewhat higher than those used in these experiments, inhibited absorption of chloride from intestinal loops. These experiments may not be directly related to our observations on inhibition of absorption of oleate by taurocholate, for Peters used ileal loops. Peters found the normal ileal taurocholate concentration to be lower than that of the duodenum.

Riegel et al. (2) found that the absorption of oleic acid was not appreciable when introduced into the intestinal loops alone, while our data indicate that appreciable absorption of sodium oleate did occur. The present results are not necessarily in disagreement with theirs, for in this work the sodium salt, which is soluble, was used. The finding of Riegel et al., that taurocholate promoted absorption of oleic acid, may have been due to the alkaline reaction of the sodium taurocholate, which might have imparted to the oleic acid a tendency to dissolve and then be more easily absorbed.

Projected experiments with desoxycholate are not reported, for it was soon evident that desoxycholate in quantities equimolar with those used in the taurocholate experiments caused severe reactions in the intestinal mucosa. Blood was found in the few samples removed after introduction of sodium desoxycholate.

Analysis of intestinal juice indicated that fatty acids were excreted regularly in small amounts, whether absorption experiments were in progress or not.

Fatty acids have been isolated from juice obtained from these loops. The results will be reported elsewhere.

SUMMARY

Sodium oleate was absorbed to an appreciable extent from jejunal loops of dogs.

Gall-bladder bile enhanced absorption of sodium oleate.

Sodium taurocholate and sodium glycocholate separately or together, in the concentrations used, failed to promote absorption of sodium oleate.

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INCREASED DEXTROSE APPETITE OF NORMAL RATS TREATED WITH INSULIN

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Previous experiments have shown that rats made hyperglycemic by removal of the pancreas had a decreased appetite for carbohydrate but an increased appetite for fat (Richter and Schmidt, 1941). On their dietary selections, blood sugar and other diabetic symptoms either diminished in intensity or disappeared altogether. Further experiments have now been undertaken to determine whether normal rats made hypoglycemic by treatment with insulin will make an analogous compensatory effort to increase their blood sugar by ingesting larger amounts of dextrose.

METHODS. Eleven male rats were kept separately in cages 10 x 8 x 13 inches, each of which contained a food cup and two graduated inverted 100 cc. bottles. The stock diet was made according to the following formula:

Graham flour.....	725 grams
Skim milk powder.....	100 grams
Casein.....	100 grams
Butter.....	50 grams
Calcium carbonate.....	15 grams
Sodium chloride.....	10 grams

Of the caloric value of this diet, carbohydrate constituted 60.1 per cent; fat, 14.8 per cent; protein, 25.1 per cent. One bottle was filled with tap water, the other with a 40 per cent solution of dextrose.

Daily records were made of the food and fluid intake, and weekly records were made of body weight.

After 10 to 20 days, when the dextrose solution and water intake had reached fairly constant levels, treatment with insulin was started. Daily injections of insulin (protamine zinc—40 units per cc.) were given subcutaneously. The initial dosage, 2 units (0.025 cc. per unit), was increased usually each day by increments of 0.4 unit over a period of 26 to 54 days until the rats died or a level of 16 units was reached.

RESULTS. *Effect of insulin treatment on dextrose appetite.* Figure 1 gives a typical record obtained from one of the 11 rats. The daily intake of the 40 per cent dextrose solution, food, and water is shown on the ordinates; age in days, on the abscissae. The record also shows the days on which insulin injections were given and the dosage. This rat was placed in the cage at an age of 73 days, and insulin injections were started 20 days later. During the pretreat-

ment period the daily intake of the dextrose solution averaged 12.9 cc. Insulin injections, started at 2.0 units per day and increased each day in steps of 0.4 unit, definitely increased the dextrose appetite when a dosage of 6.0 units was reached. Thereafter the dextrose intake closely paralleled the increase in dosage. At the termination of the 30-day treatment period, when the dosage had reached 14.0 units, the dextrose intake was 42.0 cc. That the insulin injections had a specific effect on the dextrose appetite was shown by the fact that the treatment had only a small, if any, effect on the total food intake. Water intake showed a small decrease.

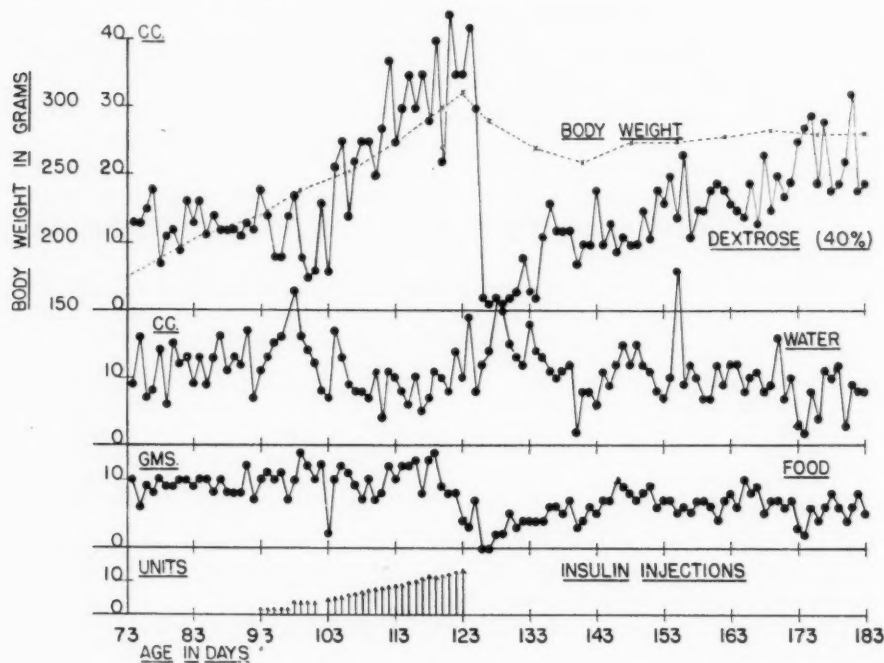


Fig. 1

Table 1 summarizes the results of the observations made with the 11 rats. The treatment period ranged from 26 to 54 days and averaged 43 days. Four rats died during treatment—26, 44, 46 and 54 days respectively after the start. The average daily dextrose intake increased from 14.6 cc. for the 10 days before treatment to 31.5 cc. for the last 10 days of treatment. This represented an average increase of 115.8 per cent (3.8 to 608.0 per cent). All 11 rats showed an increased dextrose intake. For these same 10-day periods the average food intake increased from 10.1 to 12.1 grams, or only 19.8 per cent, with variations from -27.3 to +88.4 per cent.

The total caloric intake, dextrose plus food, increased from 64.0 calories for

the 10 days preceding treatment to 98.8 calories. (See table 2.) Measured in calories per kilogram of body weight, the increase (233.3 to 274.3 calories) was relatively smaller since the animals gained so rapidly during treatment (from an average of 281 grams to 369 grams). The highest total caloric intake, as measured in calories per kilogram of body weight, is not much above the limits of the normal.

The dextrose intake increased almost in direct proportion to the dosage of the insulin injections. Even during the first 10-day period, before the daily dosage reached 5 units, the dextrose intake of most of the rats showed a small but definite increase. For the 11 rats the average daily total caloric intake reached its maximum with an average dose of 11.6 units, or 32.5 units per kilo-

TABLE 1
Insulin experiment

RAT NUMBER	AGE AT START OF EXPERI- MENT	DURATION OF TREATMENT	DOSAGE OF INSULIN	40 PER CENT DEXTROSE (CC.)			FOOD (GRAMS)		
				10 days immedi- ately preced- ing treat- ment	Last 10 days of treat- ment	Increase	10 days immedi- ately preced- ing treat- ment	Last 10 days of treat- ment	Increase or decrease
	<i>days</i>	<i>days</i>	<i>units</i>			<i>per cent</i>			<i>per cent</i>
1		44—died	2.0-12.4	18.2	31.5	73.1	10.0	13.2	32.0
2	83	46—died	2.0-16.4	13.7	26.6	94.2	10.4	15.6	50.0
3	83	31	2.0-13.2	13.1	33.4	155.0	9.1	10.1	10.9
4	83	26—died	2.0-11.5	15.2	43.0	182.9	9.4	8.4	-10.6
5	82	54—died	2.0-15.5	11.1	17.9	61.3	10.3	17.7	71.8
6	77	41	2.0-15.0	10.8	34.3	217.6	10.9	9.7	-11.0
7	77	54	2.0-16.0	8.1	31.0	282.7	13.2	14.6	10.6
8	201	50	2.0-16.0	21.5	32.9	53.0	7.8	14.7	88.4
9	201	34	2.0-12.0	5.0	35.4	608.0	12.8	9.3	-27.3
10	204	52	2.0-16.0	20.6	35.8	73.8	8.8	7.0	-20.4
11	204	45	2.0-16.2	23.7	24.6	3.8	9.0	12.4	37.7
Average.....	118	43		14.6	31.5	115.8	10.1	12.1	19.8

gram of body weight. Larger doses did not usually further increase this dextrose intake.

Effect on dextrose appetite of discontinuation of insulin treatment. The typical record in figure 1 shows that for two days after the last insulin injections the dextrose intake still remained on a very high level; then precipitately it dropped almost to zero and remained there for a few days. After that it gradually increased again. Food intake also dropped to a low level immediately after discontinuation of the insulin treatment.

Table 3 summarizes the results. It gives the average daily dextrose and food intake in calories for the last 10 days of the insulin treatment and for the 10-day period following the third day after the last injection. Only 7 rats survived the treatment; the other 4 died in convulsions apparently due to a too rapid increase

in dosage. The average daily dextrose intake decreased from 51.5 calories for the last 10 days of treatment to 6.2 calories, which represents a decrease of 88.0 per cent. Food intake decreased from 43.8 to 11.4 calories, or 74.0 per cent. Total calories decreased from 95.3 to 17.5, or 81.6 per cent.

TABLE 2

Caloric intake for the 10 day period immediately before treatment and the last 10 day period of treatment

RAT NUMBER	10 DAYS IMMEDIATELY BEFORE TREATMENT			LAST 10 DAY PERIOD DURING TREATMENT		
	Body weight	Calories (food and dextrose)	Calories (kgm. body weight)	Body weight	Calories (food and dextrose)	Calories (kgm. body weight)
	<i>grams</i>			<i>grams</i>		
1	300	69.1	230.3	354	113.2	319.8
2	235	63.5	270.2	380	105.0	276.3
3	212	57.4	270.8	293	93.8	320.1
4	215	61.9	287.9	274	102.4	373.7
5	247	59.0	238.9	374	99.4	265.8
6	253	60.9	240.7	337	93.7	278.0
7	273	65.8	241.0	373	108.0	289.5
8	356	65.6	184.2	468	103.8	221.8
9	303	59.2	195.4	354	93.8	265.0
10	348	68.2	196.0	427	85.3	199.8
11	350	73.9	211.1	428	89.0	207.9
Average.....	281	64.0	233.3	369	98.8	274.3

TABLE 3

Average daily caloric intake for rats surviving treatment

RAT NUMBER	DEXTROSE			FOOD			TOTAL CALORIES		
	Last 10 days of treatment	*10 days following treatment	Per cent decrease	Last 10 days of treatment	*10 days following treatment	Per cent decrease	Last 10 days of treatment	*10 days following treatment	Per cent decrease
3	53.4	5.6	89.5	40.4	11.2	72.3	93.8	16.8	82.1
6	54.9	3.8	93.1	38.8	13.2	66.0	93.7	17.0	81.9
7	49.6	10.9	78.0	58.4	12.0	79.5	108.0	22.9	78.8
8	49.4	7.5	84.8	54.4	7.2	86.8	103.8	14.7	85.8
9	56.6	6.1	89.2	37.2	8.4	77.4	93.8	14.5	84.5
10	57.3	1.0	98.3	28.0	13.2	52.9	85.3	14.2	83.4
11	39.4	8.3	78.9	49.6	14.4	71.0	89.0	22.7	74.5
Average.....	51.5	6.2	88.0	43.8	11.4	74.0	95.3	17.5	81.6

* Ten day period started on 3rd day after discontinuation of treatment.

MacKay and Callaway (1937) and MacKay, Callaway and Barnes (1940) have reported that protamine zinc insulin injections increased the food intake and the fat deposition in rats. Our results show that, although the total caloric intake is increased due to the increased dextrose intake, the intake of stock diet may actually be decreased. Our results confirm their findings of the sharp

decrease in food intake which occurs immediately after the cessation of the insulin injections.

Of special interest is the fact that shortly after the end of this 10-day post-treatment period the dextrose began to increase again, ultimately reaching a constant level well above that present before treatment. Food intake never regained its pretreatment level. Figure 1 shows this difference between the dextrose and food intake after the discontinuation of the treatment. For this animal in the 10-day period taken 40 to 50 days after the last injection, the dextrose intake averaged 18.0 cc., as compared to 12.9 cc. for the pretreatment period; and the food intake was 6.8 grams, as compared to the average of 9.1 grams for the pretreatment period.

Table 4 summarizes the results. It gives the average daily caloric intake for dextrose and food of the 7 rats surviving treatment for the 10-day pretreatment period, for the last 10-day treatment period, and for the 10-day period from 40 to 50 days after treatment was stopped and dextrose and food intake had at-

TABLE 4

Average daily caloric intake before, during, and after insulin treatment (7 rats)

	DEXTROSE	FOOD	TOTAL	RATIO: $\frac{\text{DEXTROSE}}{\text{FOOD}} =$
Pretreatment (10-day average).....	23.5	40.9	64.4	$\frac{23.5}{40.9} = 0.57$
Treatment period (last 10 days).....	51.4	43.8	95.2	$\frac{51.4}{43.8} = 1.17$
Post-treatment (40-50 days).....	29.9	27.2	57.1	$\frac{29.9}{27.2} = 1.10$

tained fairly constant levels. Dextrose intake increased from 23.5 calories before treatment to 51.4 during treatment, and then decreased to 29.9 after treatment, or 6.4 calories above the pretreatment level. Food intake decreased from 40.9 for the pretreatment period to 27.2 for the post-treatment period, or 13.7 calories. The total caloric intake decreased from 64.4 to 57.1 calories. The ratio of dextrose to food intake increased from 0.57 for the pretreatment period to 1.17 during treatment and to 1.10 for the post-treatment period.

Observations made on 6 control rats treated with exactly the same doses of insulin but not given access to dextrose show that the increased dextrose intake had beneficial effects on the experimental rats. Four of these 6 rats died after 5, 11, 12 and 21 days of treatment with still relatively small doses. Two were still alive after 34 days when treatment was stopped. In the experimental group only 4 of the 11 rats died and not until after 26, 44, 46 and 54 days when the dosage had increased to relatively high levels.

DISCUSSION. The present results bring further evidence to show that when the physiological means of maintaining a constant internal environment break down or are removed, as for instance after glandular disease or extirpation, the whole animal reacts toward homeostasis. Thus, rats deprived of their

adrenal glands seek salt and by virtue of their increased salt intake keep themselves alive and free from symptoms of insufficiency (Richter, 1936). Likewise, parathyroidectomized rats take calcium and as a result keep themselves alive and free from symptoms of tetany (Richter and Eckert, 1937). In the present experiments the rats made hypoglycemic by insulin treatment attempted to restore their blood sugar to its normal level by ingesting large amounts of dextrose. The fact that the rats did not eat more stock food at the same time demonstrates that they had a specific need for dextrose. Furthermore, the sharp but temporary decrease in dextrose appetite which followed the discontinuation of treatment may have indicated that for a short time the rats had a decreased need for dextrose. During this time the large amounts of fat stored up during insulin treatment must have supplied most of the animals' energy needs. This storage may be regarded as another instance of the inverse relationship that our self-selection experiments have shown almost invariably exists between carbohydrate and fat appetite. The decreased dextrose appetite may also indicate the presence of a temporary hyperglycemia. Clinically high blood sugars have been found within the first few days after removal of pancreatic adenomata (Whipple and Frantz, 1935). From the same point of view the increased dextrose appetite and lowered food appetite present after discontinuation of treatment may indicate that insulin treatment may have rested the pancreatic cells so much that they secreted more rather than less insulin. This excessive secretion may have resulted in a mild state of hyperinsulinism. Clinically it has been found that low blood sugar levels often may persist after the otherwise apparently successful removal of pancreatic tumors (Fraser, McClay and Mann, 1938; Whipple and Frantz, 1935; and West and Kahn, 1939).

SUMMARY

1. Eleven adult rats treated daily with progressively increasing doses of insulin (from 2 to 16 units per day for 26 to 54 days) all manifested a markedly increased appetite for a 40 per cent solution of dextrose. The average daily dextrose intake increased from 14.6 cc. for the last 10 days before treatment to 31.5 cc. for the last 10 days of insulin injections.
2. For the same periods the intake of stock food increased only from 10.1 to 12.1 grams, thus demonstrating the specificity of the dextrose appetite.
3. Apparently the rats made an effort to correct the lowered blood sugar by ingesting large amounts of dextrose.
4. Discontinuation of treatment caused a sharp but temporary decrease in dextrose appetite to almost zero level for several days. Food intake showed a less sharp decrease. By their appetites the rats indicated that during this time they needed less sugar.
5. Several weeks after discontinuation of insulin treatment the dextrose appetite was greater than during the pretreatment period, and food intake was lower. This was taken to indicate that a mild degree of hyperinsulinism still existed. In agreement with this, clinical experience on human beings has

shown that after removal of a pancreatic tumor low blood sugar levels may persist for long periods.

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